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The Effects of Exogenous Triiodothyronine on Reproductively Inhibited Prairie Deer Mice (*Peromyscus maniculatus bairdii*) from Laboratory Populations

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THE EFFECTS OF EXOGENOUS TRIIODOTHYRONINE
ON REPRODUCTIVELY INHIBITED PRAIRIE DEERMICE
(PEROMYSCUS MANICULATUS BAIRDII)
FROM LABORATORY POPULATIONS

A Thesis
Presented to
The Faculty of the Department of Biology
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Arts

by
Paul S. Hogg

1989

APPROVAL SHEET

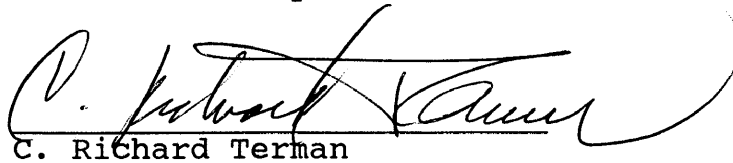
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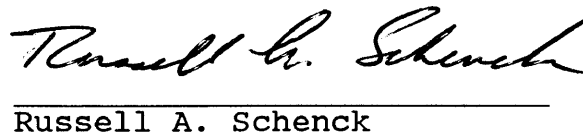

Russell A. Schenck

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ABSTRACT

This study examined the effects of exogenous triiodothyronine (T3) on reproductively inhibited prairie deermice from laboratory populations. The study had three objectives. First, to administer exogenous T3 via food and measure serum concentrations of T3 in untreated and treated animals. Second, to analyze possible gravimetric differences between the reproductive organs of untreated and treated animals. Third, to determine the reproductive status of untreated and treated animals as evidenced by histological evaluation of the gonads.

The measurement of serum T3 concentrations was based on three parameters: serum concentrations as determined by radioimmunoassay, per capita food consumption, and analysis of percent body fat. Both treatment males and females had significantly greater serum T3 concentrations than the corresponding untreated animals. Per capita food consumption increased significantly during the time of T3 administration as compared to per capita food consumption prior to T3 administration. The percent body fat of both treatment males and females was lower than the corresponding untreated animals but the difference was not significant.

Gravimetric analysis revealed a significant increase in seminal vesicle and testis weights of treatment males when compared to untreated males. Likewise, uterus and ovary weights of treatment females were significantly greater than those of untreated females.

Histological analysis of treatment and untreated male testes showed a significant increase in testis width, number of seminiferous tubules, number of cells per seminiferous tubule, and number of spermatids with acrosome formation in treatment males. A similar analysis of treatment and untreated female ovaries showed no significant differences in numbers of type six follicles, type seven follicles, atretic follicles, or corpora lutea, however, there was a significant increase in numbers of type eight follicles in treatment females.

T3 administration initiated statistically significant, but rather, limited degrees of physiological change. Based on these findings thyroid function may have a role in the reproductive inhibition exhibited by prairie deermice from laboratory populations, however, this role is probably in concert with the functions of other endocrine systems.

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INTRODUCTION

The growth of laboratory populations of prairie deermice, Peromyscus maniculatus bairdii, is limited despite the presence of surplus food and water. Population growth reaches asymptote due to either a failure of young to survive or a cessation of reproduction (Terman, 1965, 1969, 1973a, 1973b). While this phenomenon appears to be density related, it is not density dependent as demonstrated by the variability in animal numbers among asymptotic populations maintained under identical conditions (Terman, 1965, 1969, 1987).

Body weights, testis weights, and seminal vesicle weights of reproductively inhibited population males are significantly lower than the weights of reproductively capable non-population males (Terman, 1969; Bradley and Terman, 1981a, b, c; Pitman, 1983). Reproductively inhibited population females have significantly lower body weights, ovarian weights and uterine weights than reproductively capable non-population females (Terman, 1969; Bradley and Terman 1981a, c; Pitman, 1983.) In addition, inhibited population females exhibit a

significant reduction in percent body fat and relative adrenal weights when compared with reproductively capable females (Bradley and Terman 1981a,c; Cronin and Bradley, 1988).

Histologically, reproductively inhibited population animals demonstrate many differences when compared with reproductively capable animals. The cross-sectional areas of the testes, the number of seminiferous tubules, the area of seminiferous tubules and the number of spermatids with acrosomes of reproductively inhibited population males are significantly lower than reproductively capable males (Bradley and Terman, 1981c). Reproductively inhibited population females have significantly fewer Type 8 follicles and corpora lutea than reproductively capable non-population females (Bradley and Terman, 1981c). In concert with these findings are earlier reports by Terman (1973a) that reproductively inhibited population females have significantly more atretic follicles than reproductively capable females. Such histological findings may partially explain why ninety-four percent of females born into populations and surviving until ninety days of age fail to produce young (Terman, 1973a).

Many endocrine contrasts exist between reproductively inhibited population and reproductively

capable animals. In reproductively inhibited population males, FSH and serum corticosterone concentrations are significantly elevated (Bradley and Terman, 1981a, c) even though adrenocorticotrophic hormone (ACTH) concentrations do not differ significantly between reproductively inhibited population and reproductively capable males (Coppes and Bradley, 1984). Testosterone, prolactin, tetraiodothyronine (T₄), and triiodothyronine (T₃) serum concentrations have also been shown to be significantly reduced in reproductively inhibited population males (Bradley and Terman, 1981a; Pitman and Bradley, 1984; Kirkland and Bradley, 1986). Reproductively inhibited population females have significantly elevated levels of FSH and corticosterone and significantly lower levels of LH, serum prolactin, T₄, and T₃ when compared to reproductively capable females (Bradley and Terman, 1981a,c; Pitman and Bradley, 1984; Kirkland and Bradley, 1986).

The earlier work mentioned above has examined the relationships between the gravimetric, histological and endocrine characteristics exhibited by reproductively inhibited population animals. A portion of this work focused on the possible involvement of thyroid hormones (Pitman, 1983; Peebles et al., 1984). Given that both body and reproductive organ weights were reduced in reproductively inhibited population animals, such an

approach was logical since virtually all post-natal development and growth is dependent upon direct and indirect thyroid hormone mediated effects (Bernal and DeGroot, 1980; Norris, 1985, Ingbar and Woeber, 1981).

Previous studies have demonstrated a relationship between thyroid function and reproductive inhibition. Work by Pitman and Bradley (1984) established that at 1400 ± 30 minutes (on a light cycle of 14 h light, 0700-2100, and 10 h dark) serum thyroxine levels were stable for both sexes of control and reproductively inhibited population animals and serum T4 and T3 concentrations were significantly reduced in reproductively inhibited population males and females. Peebles et al. (1984) demonstrated a significant reduction in reproductively inhibited population male serum T4 but no significant reduction in reproductively inhibited population female T4 concentrations. The discrepancies between these two studies may be the result of differences in sampling times (1100 h for Peebles et al., 1984 and $1400 \text{ h} \pm 30 \text{ min}$ for Pitman and Bradley, 1984) Further Pitman and Bradley (1984) found through histological analysis of thyroid tissue that reproductively inhibited population males had a significantly decreased mean cell height and mean number of follicle cells in comparison with corresponding reproductively capable males. Reproductively inhibited population females had significantly increased follicular

area, colloidal area, and number of cells per follicle compared with reproductively mature females (Pitman and Bradley, 1984). They concluded that these results suggested secondary hypothyroidism in males and primary hypothyroidism in females (Pitman and Bradley, 1984).

Peebles et al., (1984) studied the effects of injected thyroxine on reproductively inhibited population males and found a significant increase in testes weights but no significant increase in seminal vesicle weights. At the histological level, significant increases in (1) the width of testes cross-sections, (2) the mean number of seminiferous tubules, (3) the mean number of cells per seminiferous tubule, and (4) the mean number of spermatids with acrosomes were noted in T4 treated reproductively inhibited population males. These results appeared to show a recovery effect brought about by T4 injection. However, the changes may have been partly due to repeated daily handling of the injected males and not just a direct result of pharmacological effects produced by the T4.

Past work by Peebles et al., (1984) showed that injection of T4 may have caused some degree of reproductive recovery in reproductively inhibited population animals. The current study examined this phenomenon further by administering the more biologically

active thyroid hormone, L-3,5,3'-triiodothyronine (Calbiochem, Behring Diagnostics). This study was designed to meet three objectives. The first was to administer T3 in food and determine if treated animal serum concentrations of T3 were elevated compared with untreated animal serum T3 levels. The second was to analyze possible gravimetric differences between untreated and treated animals' reproductive organs. The third objective was to determine the reproductive status of untreated and treated animals as evidenced by histological evaluation of the gonads.

MATERIALS AND METHODS

Founding of Populations

The animals used in this study were prairie deermice, Peromyscus maniculatus bairdii, obtained from two laboratory populations previously founded from a laboratory colony (Creigh and Terman, 1988). The laboratory colony was outbred with bisexual pairs housed individually in plastic cages (28 x 12.7 x 15.4 cm) with wood shavings as bedding (Terman, 1965). Both food (Agway Prolab 3000) and water were provided in surplus. Young were weaned at 21 days of age and placed with sibs of the same sex. Four proven bisexual pairs of colony adults from eight different litters (100-150 days of age) were placed in each of five population enclosures (see Animal Maintenance). Any young born within twenty-one days of founding the population was removed to insure that all young produced were a result of impregnation within the population context. The two populations in this study were referred to as population "three" and "four" since this was their original designation in the earlier studies (Creigh and Terman, 1988).

Animal Maintenance

Population animals were kept in 1.5 m diameter by 0.75 m deep circular enclosures constructed of corrugated aluminum. The floor of each enclosure was covered with wood shavings which were not changed throughout the period of animal maintenance or during the T3 feeding period. Population animals were provided with surplus food (Agway Prolab 3000) and water. They were kept in a light cycle of 14 hours light (0700 - 2100 h) and 10 hours dark. Illumination was provided by four 40W fluorescent tubes which provided 15 ft-c at the level of the floor (Bradley and Terman, 1981c). Throughout the study laboratory temperatures were maintained within a range of 20^o - 30^o C and air was exchanged five to ten times per hour.

Scheduled population inspections were made every two weeks except during the T3 feeding period when inspections were made every week. During these inspections, notations were made of all vaginally perforate or imperforate and pregnant or lactating females, and all males with scrotal or non-scrotal testes. Body weights of all animals were recorded starting two months prior to and then throughout the T3 feeding period. Any surviving young born into the

populations were identified by toe clipping and their reproductive status and body weights were monitored during subsequent inspections.

Experimental Design

The overall experimental design involved administering T3 to two laboratory populations, one at asymptote and another with only limited growth (two litters produced 110 days prior to the experiment). During the period from day 0 (day when feeders were installed) to day 21 (day when T3 administration began) the feeders contained only powdered food. This was designated the "untreated" period. During this time the feeders were weighed at 1400 h \pm 30 min every day to obtain an estimate of daily population food consumption. On day 18 the untreated animals were removed. Untreated animal's ages ranged from 231 to 1030 days. They were selected on the basis of continuous histories of non-scrotal testes or imperforate vaginae and no pregnancy. T3 administration began on days 21 and 22 for populations three and four respectively. T3 administration continued until day 56 for population three and day 57 for population four. At these times the T3-treated animals were removed. Treated animals were pre-selected in the

age range of 206 to 1153 days old and on the basis of continuous histories of non-scrotal testes or imperforate vaginae and no pregnancies prior to T3 feeding. During the treatment period, the feeders were also weighed at 1400 h \pm 30 min each day to obtain an estimate of daily population food consumption.

The hormone was administered in food to avoid complication of long-term repeated handling. Also, food intake is related to body weight and therefore dosages are proportional to body mass. The preparation of the T3/food mixture was a multi-step process patterned after a procedure used by Cogburn (1988). First, 2.5 mg of powdered T3 was thoroughly mixed with 200 g of powdered dextrose in a Waring blender. Then 20.2 g of the resultant mixture was blended with 980 g of pre-powdered food (Agway Prolab 3000). The final T3/food mixture contained a dose of 250 ng of T3 per gram food. Since the T3/food mixture was in powdered form, a special feeder was built to provide the population animals with easy access to the T3/food mixture and yet prevent contamination by excrement and bedding which might alter the daily weighings of the feeder to determine food consumption. Feeders were placed in the center of each of the two population enclosures. Water was available ad libitum.

Analysis of Serum Triiodothyronine Concentrations

Three parameters were chosen that would provide quantitative as well as physiological evidence of elevated serum T3 levels. The first parameter was a quantitative measurement of serum T3 concentrations using radioimmunoassay techniques. The second was the measure of total and calculated per capita food consumption of the two populations prior to and during hormone administration. The third was the analysis of percent body fat which would give an indirect measure of metabolic rate and thus T3 levels.

Radioimmunoassay of Triiodothyronine

Blood samples were obtained from the dorsal abdominal aorta by laparotomy while animals were under diethyl ether anaesthesia. All animals were sampled within thirty minutes of initial disturbance (Dohler et al., 1977). The sampling time chosen was 1400 h \pm 30 minutes based on previous work by Pitman and Bradley (1984). Blood samples were centrifuged (Beckmann Microfuge) at 9000 x g for two minutes immediately after the collection process was complete. The sera samples were stored at approximately ^o-65 C until they were assayed.

T3 was assayed using a Gamma Coat [I-125] T3 RIA kit produced by Baxter Travenol Diagnostics, Inc. (a division of Dade Pharmaceuticals). This radioimmunoassay utilizes a competitive binding technique in which T3 present in P. maniculatus serum competes with a radioactive tracer(I-125 labelled T3) for a limited number of binding sites on T3 antibodies (produced from rabbit serum) coating the lower inner wall of the assay tube. The amount of I-125 T3 tracer which will bind to the assay tube (determined by measuring the net radioactivity with a gamma counter) is inversely proportional to the concentration of T3 in the P. maniculatus serum sample. Therefore, by using T3 serum standards of known concentrations, a standard curve can be developed from which unknown concentrations of P. maniculatus serum T3 can be derived. The kit was known to measure total serum T3 because 8-anilino-1-naphthalenesulfonic acid (ANS), a compound which frees T3 from its binding protein, was present in the I-125 tracer solution.

Due to the number of P. maniculatus serum samples that were tested in duplicate, it was necessary to make two RIA runs. It was also necessary to generate P. maniculatus serum serial dilution curves in order to verify the parallel binding properties between the P. maniculatus serum and the human standards supplied in the

kit. These P. maniculatus serum serial dilution curves were produced from a P. maniculatus serum pool of adult colony animals (age 90 - 120 days).

The RIA procedure first involved allowing all reagents(I-125 T3 tracer/buffer solution, T3 serum blanks, T3 serum standards, and T3 control serums) and P. maniculatus serum samples to reach ambient temperature. After warming, all reagents were mixed thoroughly for 30 seconds without foaming on a vortex mixer set at low speed. Next 50 microliters of T3 serum blank, T3 serum standards, T3 control serum and P. maniculatus serum were added to the appropriate duplicate assay tubes which had been labelled earlier. Following this, 1.0 ml of I-125 T3 tracer/buffer reagent was added to each tube and the tube was then gently vortexed and covered with parafilm. The addition of all reagents and P. maniculatus sera took no more than fifteen minutes between the first and last tube using an automatic pipette (Micromedic Systems Model 25004). All tubes were incubated in a water bath at $37 \pm 2^{\circ}\text{C}$ for 1 hour. After incubation tubes were decanted and allowed to drain in an inverted position for 5 minutes while in contact with absorbent paper to insure that the removal of all solution was complete. The tubes were then counted using a Beckmann Biogamma II counter set to count

each tube up to 40,000 counts or 20 minutes, whichever came first.

Counts per minute (CPM) for the standard and P. maniculatus sera were logit transformed. The logit transformed counts for the T3 sera standards were plotted against \log_{10} transformed T3 serum standard

concentrations on a linear scale. Using the same method the logit transformed CPM's for the P. maniculatus serum serial dilution were plotted against \log_{10} microliters P.

maniculatus serum. Linear least squares regression lines were fit to both the standards and P. maniculatus serum serial dilution plots. The regression coefficients (slopes) of these lines were then tested for equality (Sokol and Rohlf, 1985) to verify parallel binding properties between the P. maniculatus serum and the human standards supplied with the kit. Since two radioimmunoassay runs were performed, the regression coefficients of the two standard curves were also tested for equality. Subsequently, the T3 concentrations of the untreated and treated animals of both sexes were derived from these standard curves.

Measurement of Overall and Per Capita Food Consumption

A daily per capita estimation of food consumption was made by dividing daily consumption by the number of animals in the population. The values used to calculate mean per capita food consumption during the untreated period were obtained from the food consumption plateau of days 0 through 21 (see Figure 6). Likewise, the values used to calculate mean per capita food consumption during the T3 feeding period were obtained from the food consumption plateau of days 26 through 57 (see Figure 6).

Analysis of Percent Body Fat

Body fat analysis was conducted according to the method described by Cronin and Bradley (1988). A total of 24 animals were analyzed from populations three and four combined. Within each population three animals of each sex from each treatment group were chosen based on mean body weights. After removal of the reproductive organs for gravimetric and histological analysis, selected animals were frozen at -65°C for 1 hour and then lyophilized for 72 hours at -120°mTorr and -30°C . The animals were then weighed and individually powdered using a Waring blender. The powdered material of each

animal was then divided equally into three packets constructed of pre-weighed Watmann #1 filter paper (the individual packets were secured using a paper clip).

Each packet was then lyophilized at -120 mTorr and -30^o C for 48 hours to insure a constant dry weight. After weighing, packets were then placed in a Soxhlet extractor with diethyl ether for 24 hours. The packets were then once again lyophilized for an additional 48 hours at -120 mTorr and -30^o C. Final weights of each packet were obtained and subtracted from the individual packet weights prior to extraction. These individual differences for each packet were summed and represented the percentage of body fat in the total dry weight (total dry weight being the powdered dry weight of the whole animal before extraction).

Gravimetric Analysis of Reproductive Organs

During the population inspections immediately prior to the removal of the untreated and the treated animals, a non-toxic UV sensitive dye (Raytech Industries) was applied to the tails of selected animals. This dye fluoresced under illumination with a 40 W UV fluorescent tube thereby allowing positive identification and rapid sampling of the selected animals. As mentioned previously, animals were sampled at 1400 h \pm 30 minutes

and sampling was completed within 30 minutes of initial disturbance. After blood samples and body weights were taken, the animals were placed in ten percent buffered formalin for at least two weeks and no more than forty days before gross dissection. Upon gross dissection the testes, seminal vesicles, ovaries, uteri, adrenals and surrounding tissue were removed and stored in a buffer solution (42 ml 0.2M Na PO₂ / 8ml 0.2M KH PO₂) of pH 7.42.

After fine dissection, the testes, seminal vesicles, ovaries, and uteri were allowed to briefly dry on paper towels and then weighed to the nearest 0.1 mg using either a Sartorius MP8 analytical balance interfaced with a Commodore computer or a Fischer Scientific XA analytical balance.

Histological Analysis of Gonads

After the gravimetric analysis of the reproductive organs was complete a selection process was begun to determine which testes and ovaries would be examined histologically. It was decided that gonads of individuals representing the two extremes of the gravimetric range along with the mean and the two closest values to the mean (one above and one below) would be selected. This criterion was used for selection of both testes and ovaries from treated animals, however, it was

not used in regard to untreated animals since the gonads of all these animals were analyzed.

Both testes and ovaries were initially dehydrated in solutions of increasing ethanol concentration. Following dehydration the tissues were infiltrated with JB-4 infiltration solution and subsequently embedded in JB-4 embedding media (a glycol methacrylate compound produced by Polysciences, Inc.). Because of the limitations of JB4 in regards to serial sectioning the previously embedded ovaries were embedded a second time in Histoiresin (also a glycol methacrylate produced by Cambridge Instruments, Inc.). Both testes and ovaries were sectioned at 4.5 μ m and stained with toluidine blue. Tissue was evaluated at magnifications of 100x and 400x using a Zeiss microscope. Selected tissues were photographed through a Zeiss Photomicroscope II using Kodak Panatomic-X film.

The analysis of testis histology was based on four parameters: the testis width along the major axis (transect), the number of seminiferous tubules (with a minor/major axis ratio >0.85) along the transect, the number of cells along the major axis of selected seminiferous tubules, and the number of spermatids with acrosome formation along the major axis of selected seminiferous tubules. These parameters were determined

in the following manner. The major and minor axis of the testes cross-sections were measured using an optical micrometer to find the cross-section with the largest major axis. Subsequently a transect line was established along the major axis of the largest cross-section using the optical micrometer center line (Peebles, Painter, and Bradley, 1984). The number of seminiferous tubules (with a minor/major axis ratio >0.85) touching this transect were recorded. Within these selected seminiferous tubules the number of cells (including spermatids with acrosome formation) along the major axis of the seminiferous tubule were recorded. A separate observation was made of the number of spermatids with acrosome formation along the major axis of the seminiferous tubule.

The analysis of ovary histology followed criteria established by Pederson and Peters (1968). The number of atretic, Type 6, Type 7, and Type 8 follicles, as well as the number of corpora lutea of both ovaries from each female were recorded.

Statistical Methodology:

The statistical analysis was designed to test for significant differences between the measured parameters of untreated and T3 treated animals. Initially the

assumptions of ANOVA and linear regression were addressed. If the raw data or log transformed data satisfied these assumptions a linear regression analysis was performed in order to determine if there was a relationship between the measured parameter and age, and the measured parameter and body weight. Subsequently a two-way ANOVA design was performed which tested for differences across populations and treatments. If this two-way design yielded a significant interaction term then the analysis was subdivided into individual one-way ANOVAs across populations and treatments. If there was a significant difference between populations then means and statistics for each population were reported separately, otherwise the two populations were combined.

If the assumptions of ANOVA and regression were not satisfied by the raw data or log transformed data, then Spearman's correlation coefficients were used to test for significant correlation between the measured parameter and age, and the measured parameter and body weight. The raw data were then analyzed using Mann-Whitney U tests to test for differences across populations and treatments.

A separate correlation analysis was performed using Spearman's coefficient to test for significant relationships between T3 concentrations and all measured parameters. This analysis divided study animals

according to sex and within this division looked at untreated animals, treated animals, and all animals combined (Appendices 1 through 4).

Linear regression analysis was performed to test for the significance and linearity of the P. maniculatus serum serial dilution and T3 standard curves. Subsequently, an F-test was carried out to check for the equality of regression coefficients (slopes) between P. maniculatus serum serial dilutions and T3 standard curves (Appendix 5). This test was also used to determine if there was a significant difference between the slopes of the two standard curves (from radioimmunoassay runs 1 and 2).

A significance level of less than 0.05 was utilized in all statistical analyses.

RESULTS

Analysis of Serum Triiodothyronine Concentrations

There was not a significant difference between the regression coefficients (slopes) of the P. maniculatus serum serial dilution and the T3 standard curves (Figures 1 and 2). There was also no significant difference between the regression coefficients (slopes) of the T3 standard curves from radioimmunoassay runs 1 and 2 (Figure 3). The mean serum T3 concentration in treated males and females was highly significantly ($P < 0.001$) increased when compared with untreated males and females (Tables 3 and 4; Figure 4 and 5).

The mean per capita food consumption in population three was significantly ($P < 0.0001$) greater than population four both prior to and during T3 administration (Figure 7). The mean per capita food consumption of both populations was significantly ($P < 0.0001$) increased during T3 administration as compared

with the period prior to hormone administration (Figure 7).

Linear regression analysis showed no significant relationship between percent body fat and age or percent body fat and body weight for any animals in the study. Mean percent body fat in treated males tended to be reduced ($P=0.146$) in comparison with untreated males (Table 1 and Figure 8). Mean percent body fat in treated females was also tended to be reduced ($P=0.089$) in comparison with untreated females (Table 2 and Figure 9).

Although no quantitative data were obtained concerning behavioral changes, there was an observable difference in the interactions of animals in both populations during T3 administration. During the light cycle when the tendency is for animals to huddle, it was observed that the huddling behavior was disrupted.

Gravimetric Analysis of Reproductive Organs

Linear regression analysis showed no significant relationship between testis or seminal vesicle weights and age, or body weight in either untreated or treated

males. Mean testis and seminal vesicle weights of treated males were significantly ($P < 0.05$) greater than untreated males (Table 3 and Figure 10 and 12).

Linear regression analysis showed no significant relationship between ovary or uterus weights and age, or body weight in either untreated or treated females. The mean ovary and uterus weights of both untreated and treated females in population three were found to be significantly ($P < 0.05$) larger than the respective ovary weights of both untreated and treated females in population four (Table 4, and Figures 11 and 13). The mean ovary weights of treated females from both populations were significantly ($P < 0.05$) larger than the corresponding values for untreated females from both populations (Table 4 and Figure 11).

Histological Analysis of Gonads

The mean testis width of treated males was significantly ($P < 0.01$) larger than untreated males (Table 5 and Figure 14). The mean number of seminiferous tubules (with minor/major axis ratio > 0.85) on the transect was significantly ($P < 0.01$) elevated in treated males compared with untreated males (Table 5 and Figure 15). The mean number of cells per seminiferous tubule

and the mean number of spermatids with acrosome formation per seminiferous tubule in treated males were significantly ($P < 0.01$) greater than in untreated males (Table 5 and Figures 16 and 17).

Among untreated and treated females, there were no significant differences in the mean number of atretic, Type 6 or Type 7 follicle (Table 6). However, treated females from population three had significantly ($P < 0.01$ and $P < 0.05$ respectively) more atretic and Type 7 follicles than treated females from population four. There was a significant ($P < 0.05$) increase in the mean number of Type 8 follicles in treated females compared with untreated females in both populations (Table 6, and Figures 18 and 19). There was not a significant difference in the mean number of corpora lutea between untreated and treated females in both populations (Table 6; Figure 18 and 19).

TABLE 1

Age, Body Weight, Testis Weight, Seminal Vesicle Weight, Dry Body Weight,
Percent Body Fat, and Total Serum Triiodothyronine, in Untreated and Treated Males.
(values are mean \pm SEM)

Treatments	Age (days)	Body Weight (g)	Testis Weight (mg)	Seminal		Serum	
				Vesicle Weight (mg)	Dry Body Weight (g)	Percent Body Fat (%)	Triiodo- thyronine (ng/ml)
Untreated Males (n=6)	484 \pm 85	17.56 \pm 1.17	33.3 \pm 9.5	3.1 \pm 0.8	7.25 \pm 0.6	18.6 \pm 2.8	0.64 \pm 0.03
Treated Males (n=6)	575 \pm 106	18.08 \pm 0.88	97.2* \pm 29.2	15.3n.s. \pm 7.6	5.74 \pm 0.3	13.2 n.s. \pm 1.2	10.28** \pm 0.61

*P<0.05, **P<0.01, and n.s.=not significant with respect to corresponding controls

TABLE 2

Age, Body Weight, Ovary Weight, Uterus Weight, Dry Body Weight, Percent Body Fat, and Total Serum Triiodothyronine in Untreated and Treated Females.
(values are mean + SEM)

Treatment	Age (days)	Body Weight (g)	Ovary Weight (mg)	Uterus Weight (mg)	Dry Body Weight (g)	Percent Body Fat (%)	Serum Triiodo- thyronine (ng/ml)
Untreated Females (n=6)	665 +124	15.06 +1.07	4.2 +1.1	3.4 +0.3	5.9 +0.5	16.9 +0.7	0.68 +0.05
Treated Females (n=6)	728 +133	16.35 +1.21	5.1 n.s. +1.7	4.7 n.s. +0.6	5.7 +0.5	11.7 +1.1	8.37* +0.66

*P<0.01 and n.s.=not significant in respect to corresponding controls

TABLE 3

Age, Body Weight, Serum Triiodothyronine, and Testis and Seminal Vesicle Weights in Untreated and Treated Males (values are mean \pm SEM).

Treatments	Age (days)	Body Weight (g)	Serum Triiodo- thyronine (ng/ml)	Testis Weight (mg)	Seminal Vesicle Weight (mg)
Untreated Males (n=10)	500 \pm 61	16.88 \pm 0.89	0.64 \pm 0.03	35.5 \pm 6.8	2.6 \pm 0.5
Treated Males (n=20)	632 \pm 64	18.17 \pm 0.63	10.09** \pm 0.45	71.8* \pm 11.6	17.1* \pm 7.5

*P<0.05, **P<0.001 with respect to corresponding controls.

TABLE 4

Age, Body Weight, Serum Triiodothyronine, and Ovary and Uterus Weights in Untreated and Treated Females (values are mean \pm SEM).

Treatments	Age (days)	Body Weight (g)	Serum Triiodo- thyronine (ng/ml)	Ovary Weights (mg)	Uterus Weights (mg)
Untreated Females					
Pop. 3 (n=4)	529 \pm 145	15.5 \pm 1.5	0.62 \pm 0.06	4.0 \pm 0.6	3.6 \pm 0.6
Pop. 4 (n=5)	682 \pm 157	14.1 \pm 0.6	0.72 \pm 0.05	3.2 \pm 1.4	3.0 \pm 0.1
Treated Females					
Pop. 3 (n=17)	633 \pm 75	15.6 \pm 0.38	10.12** \pm 0.78	6.0* \pm 0.7	7.6** \pm 2.5
Pop. 4 (n=7)	489 \pm 121	16.3 \pm 1.1	8.72** \pm 0.39	5.1* \pm 1.4	7.0** \pm 1.2

*P<0.05 and **P<0.001 with respect to corresponding controls.

TABLE 5

Age, Body Weight, Serum Triiodothyronine, Width of Testis Cross Section, Number of Seminiferous Tubules (a/b ratio of >0.85), Number of Cells per Seminiferous Tubule on Transect, and Number of Spermatids with Acrosome Formation per Seminiferous Tubule on Transect in Untreated and Treated Males (values are mean \pm SEM).

Treatments	Age (days)	Body Weight (g)	Serum Triiodo- thyronine (ng/ml)	Width of Testis Cross section (u)	Number of Seminiferous Tubules with (a/b > 0.85)	Number of Cells per Tubule on Transect	Number of Spermatids with Acrosome Formation
Untreated Males (n=10)	500 +61	16.8 +0.8	0.64 +0.03	1959.6 +100.3	5.2 +0.3	7.4 +0.4	0.4 +0.2 [3]
Treated Males (n=10)	667 +95	19.4 +1.0	9.38** +0.52	2617.1* +137.1	6.5* +0.4	10.3* +0.4	2.5* +0.5 [9]

*P<0.01 and **P<0.001 with respect to corresponding controls
Values in parentheses represent numbers of animals studied.

Values in brackets indicate numbers of animals possessing the structure.

TABLE 6

Age, Body Weight, Serum Triiodothyronine, Number of Atretic
Follicles, Number of Type 6 Follicles, Number of Type 7
Follicles, Number of Type 8 Follicles, and Number of Corpora Lutea
in Untreated and Treated Females (values are mean \pm SEM).

Treatment	Age (days)	Body Weight (g)	Serum Triiodo- thyronine (ng/ml)	Number of Atretic Follicles	Number of Type 6 Follicles	Number of Type 7 Follicles	Number of Type 8 Follicles	Number of Corpora Lutea
Untreated Females (n=9)	614 \pm 105	14.7 \pm 0.7	0.68 \pm 0.04	43.3 \pm 3.2	2.8 \pm 0.7	0.7 \pm 0.3	0.0 \pm 0.0	0.0 \pm 0.0
				[9]	[8]	[8]	[0]	[0]
Treated Females (n=10)	609 \pm 101	16.4 \pm 0.8	9.21** \pm 0.86	38.1n.s. \pm 2.7	3.8n.s. \pm 0.9	1.5n.s. \pm 0.4	0.8* \pm 0.4	1.7n.s. \pm 0.9
				[10]	[8]	[7]	[4]	[3]

*P<0.04, **P<0.001, n.s.=not significant with respect to corresponding controls
Values in parentheses represent numbers of animals studied.
Values in brackets indicate numbers of animals possessing the structure.

Figure 1: Standard curve and P. manipulatus serum serial dilution curve for radioimmunoassay run number one

Figure 2: Standard curve and P. manipulatus serum serial dilution curve for radioimmunoassay run number two

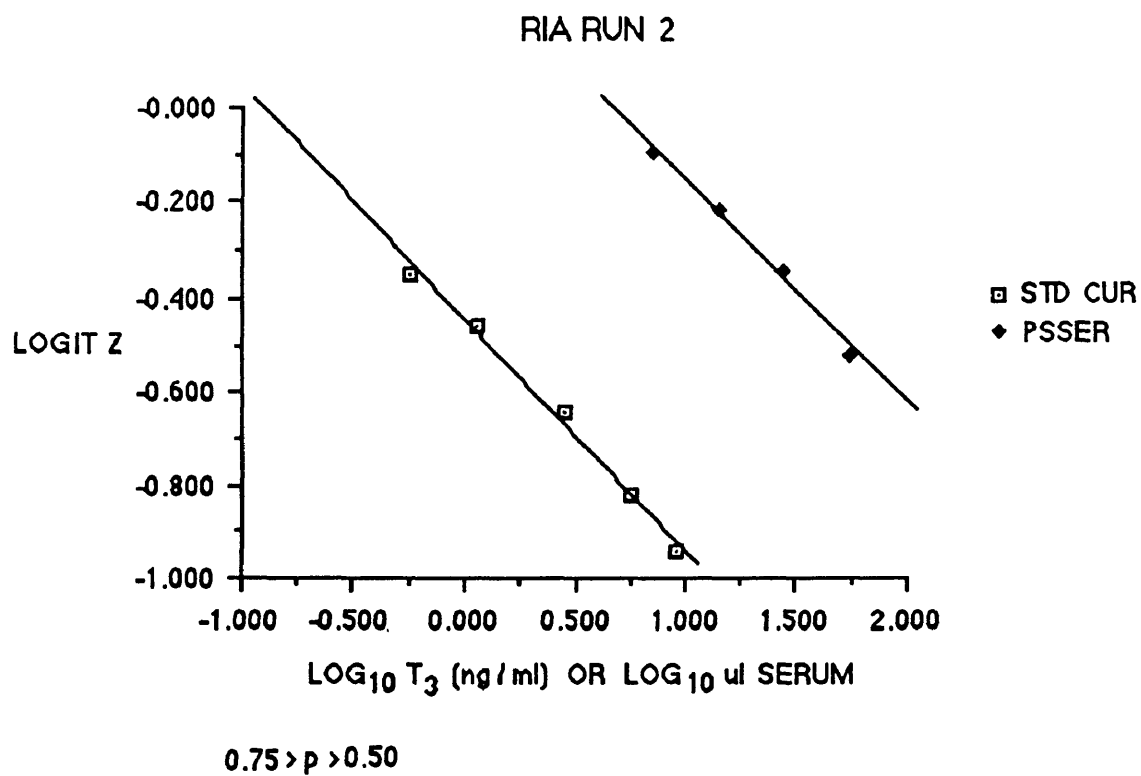
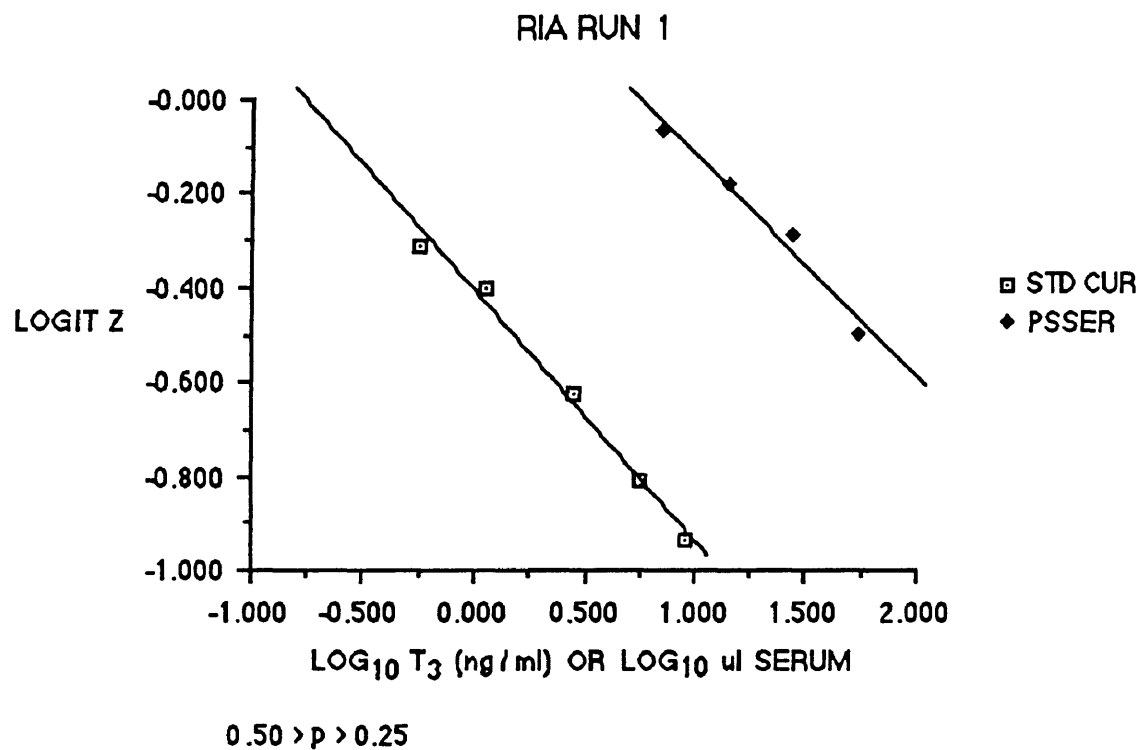
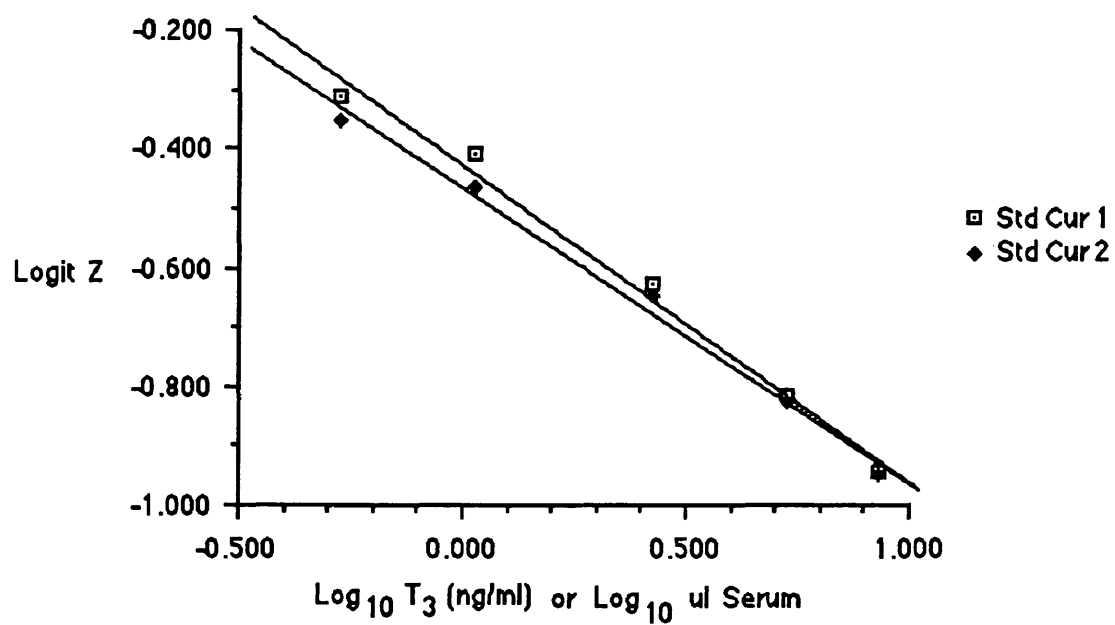


Figure 3: Standard curves for radioimmunoassay runs
number one and two

RIA RUN 1 / RIA RUN 2

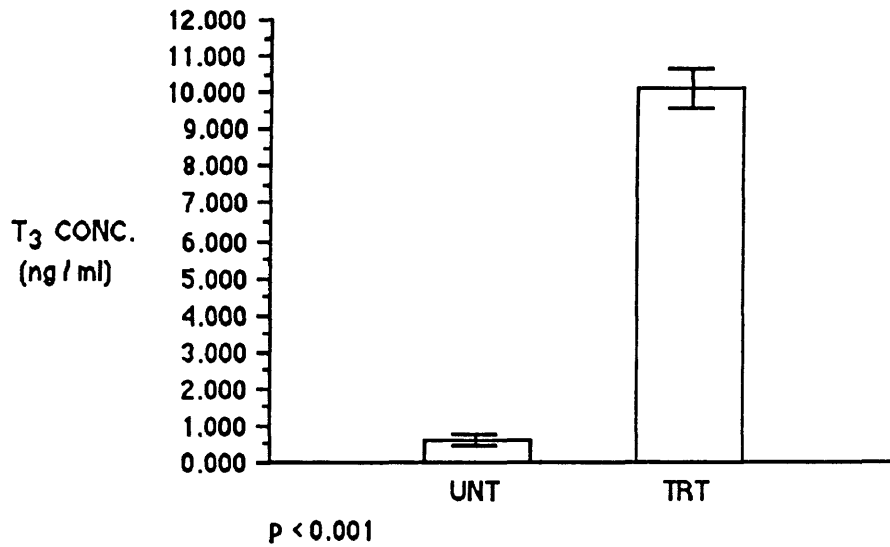


$0.50 > p > 0.25$

Figure 4: Mean blood serum concentrations of triiodothyronine in untreated and treated males from populations three and four combined. Error bars represent \pm S.E.M

Figure 5: Mean blood serum concentrations of triiodothyronine in untreated and treated females from populations three and four combined. Error bars represent \pm S.E.M

MEAN BLOOD SERUM T₃ CONCENTRATION
IN MALES



MEAN BLOOD SERUM T₃ CONCENTRATION
IN FEMALES

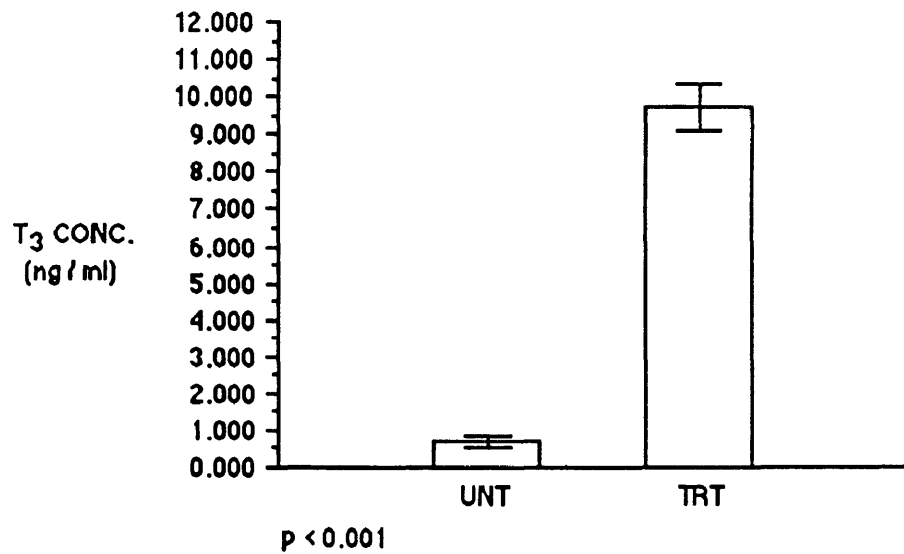


Figure 6: Per capita food consumption of populations three and four before and during triiodothyronine administration

Figure 7: Mean per capita food consumption of populations three and four before and during triiodothyronine administration.
Error bars represent \pm S.E.M.

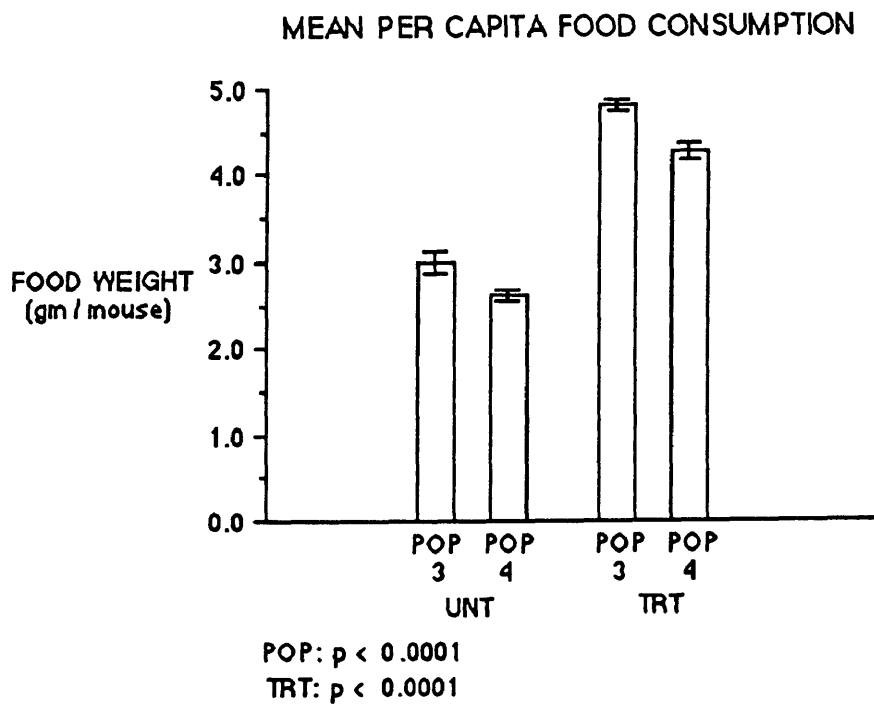
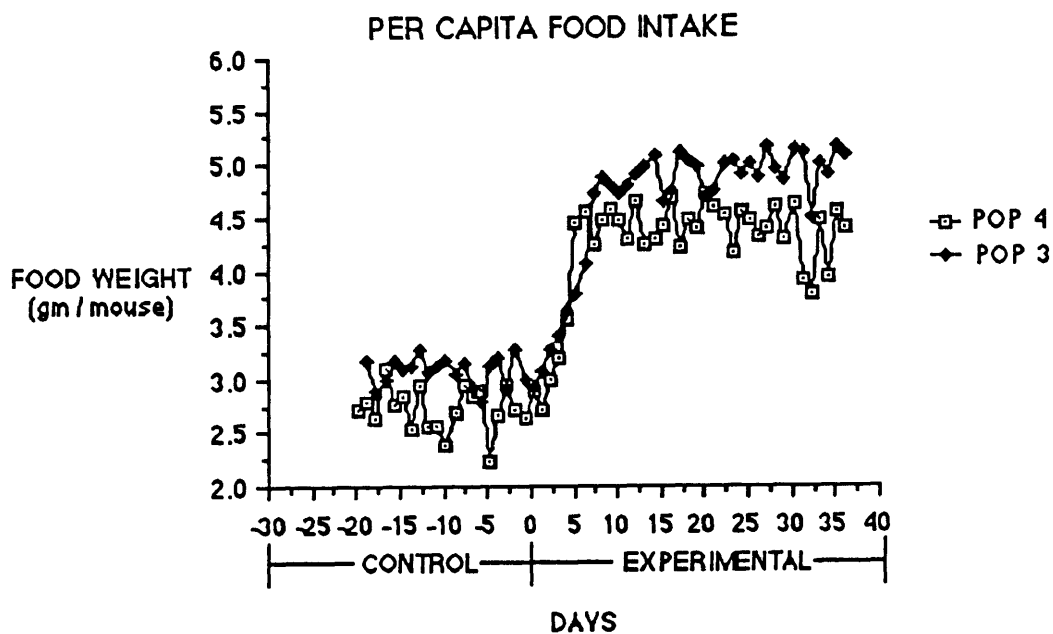
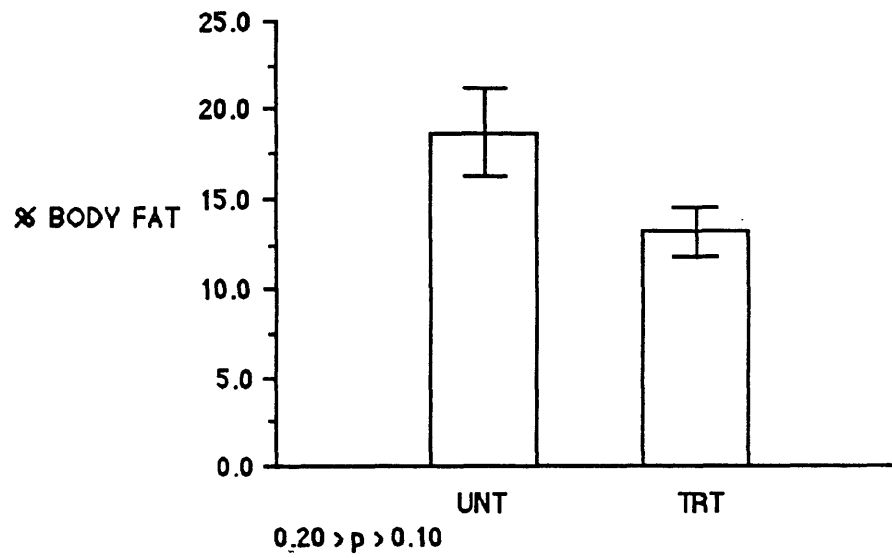


Figure 8: Mean percent body fat of untreated and treated males of populations three and four combined. Error bars represent \pm S.E.M

Figure 9: Mean percent body fat of untreated and treated females of populations three and four combined. Error bars represent \pm S.E.M

MEAN PERCENT BODY FAT IN MALES



MEAN PERCENT BODY FAT IN FEMALES

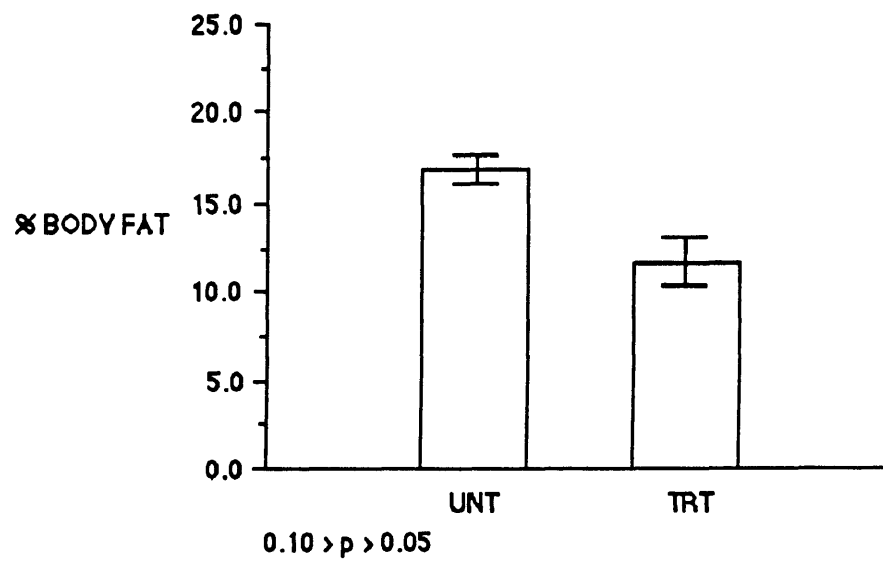
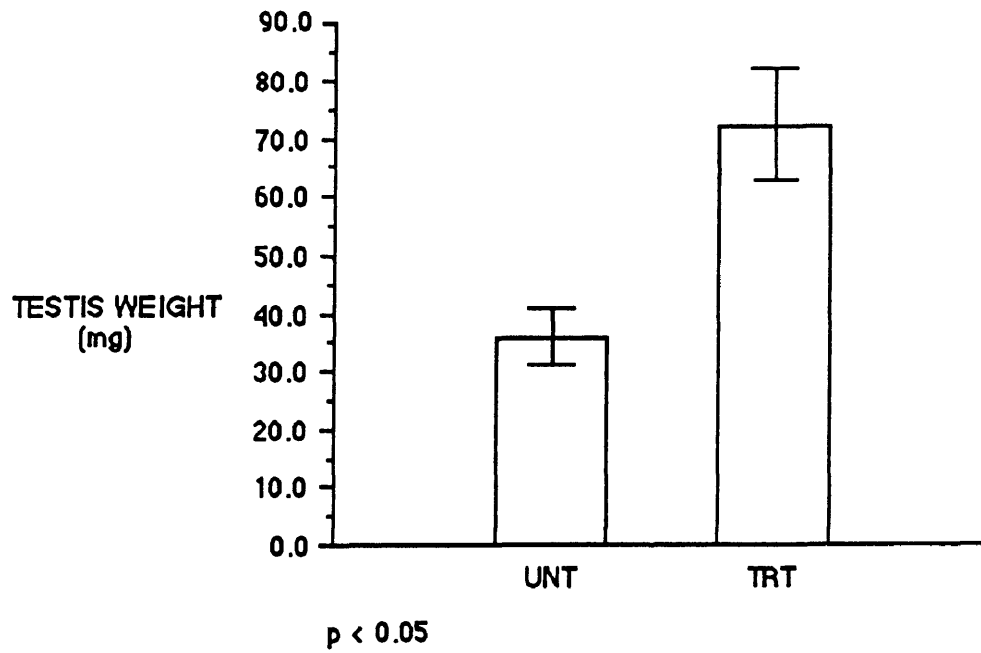


Figure 10: Mean testis weights of untreated and treated males of populations three and four combined. Error bars represent \pm S.E.M

Figure 11: Mean ovary weights of untreated and treated females of populations three and four. Error bars represent \pm S.E.M.

MEAN TESTIS WEIGHTS



MEAN OVARY WEIGHTS

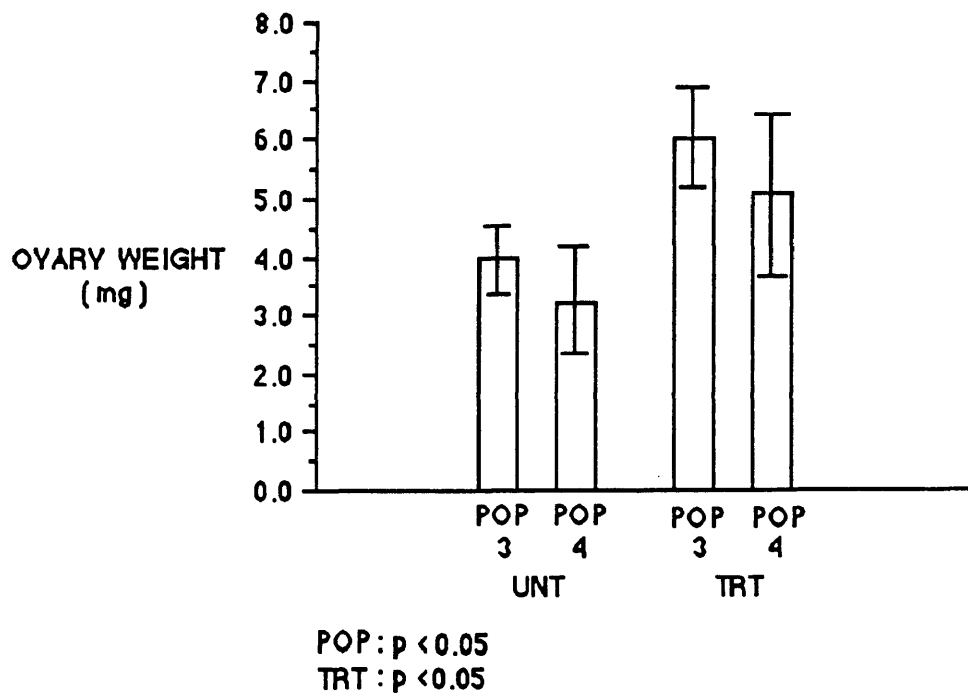


Figure 12: Mean seminal vesicle weights of untreated and treated males of populations three and four combined. Error bars represent \pm S.E.M.

Figure 13: Mean uterus weights of untreated and treated females of populations three and four combined. Error bars represent \pm S.E.M.

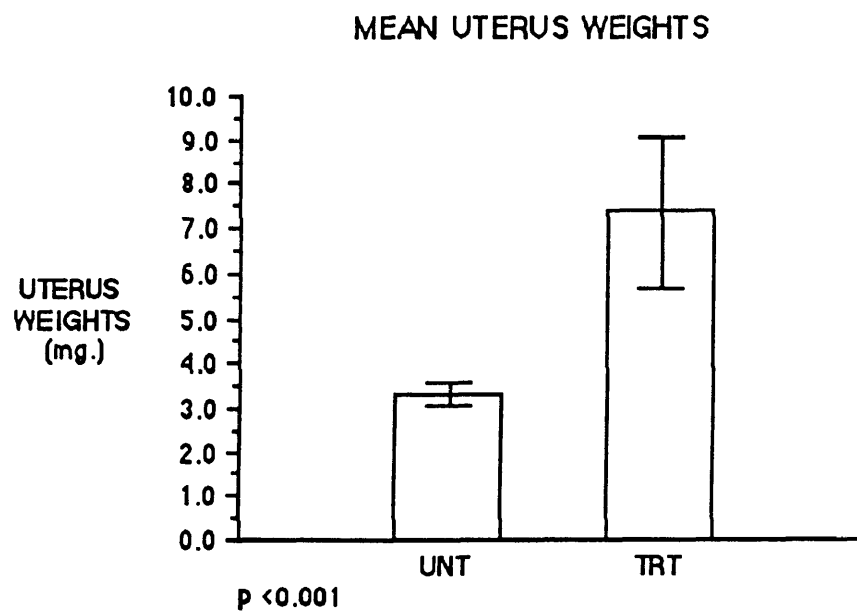
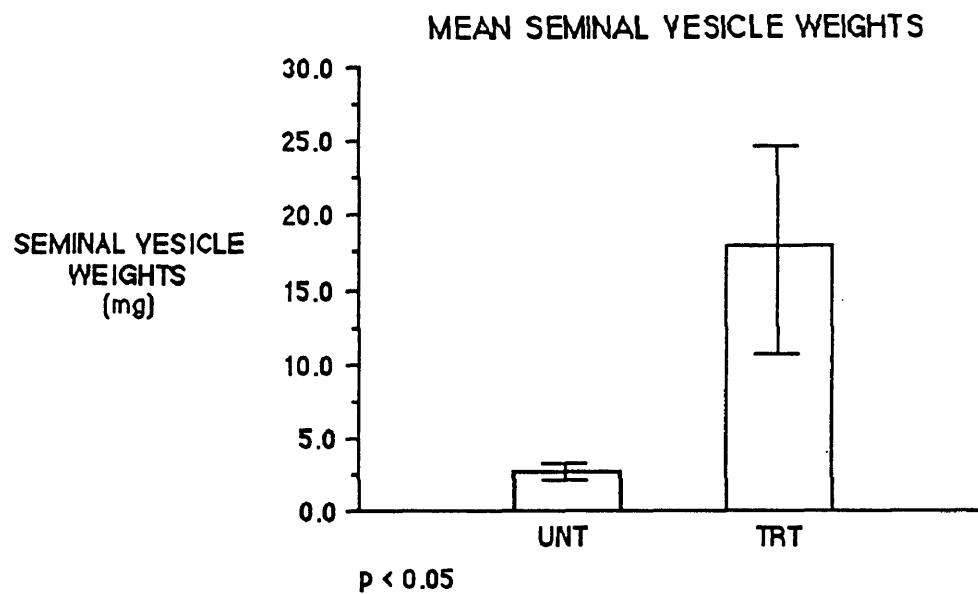
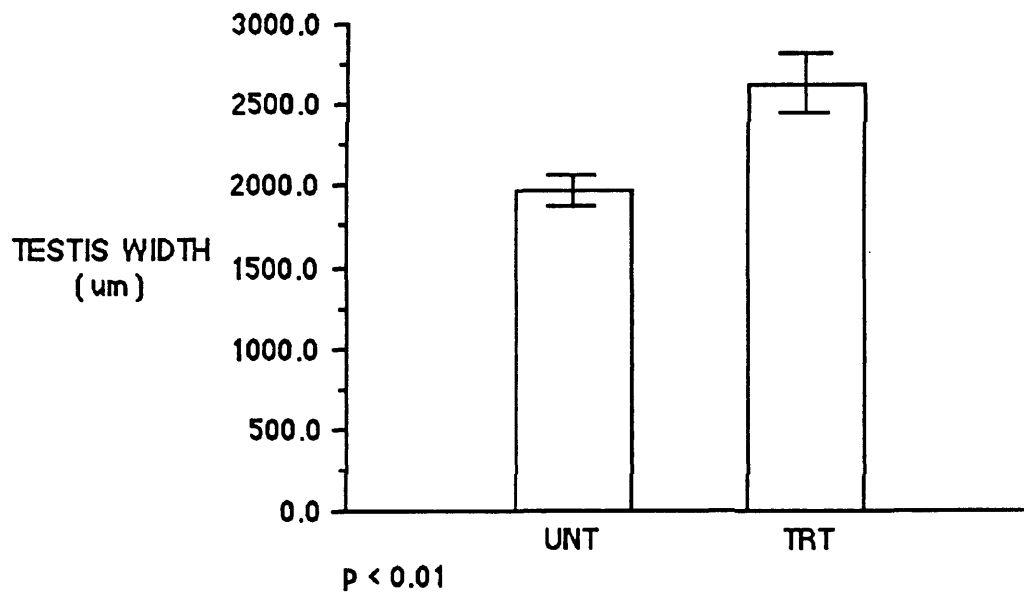


Figure 14: Mean testis width (along major axis transect)
of untreated and treated males
of populations three and four combined.
Error bars represent \pm S.E.M.

Figure 15: Mean number of seminiferous tubules
(with minor/major axis ratio >0.85) along the
transect. Populations three and four are
combined. Error bars represent \pm S.E.M.

MEAN TESTIS WIDTH ALONG TRANSECT



MEAN NUMBER OF SEMINIFEROUS TUBULES (WITH MINOR / MAJOR AXIS > 0.85)

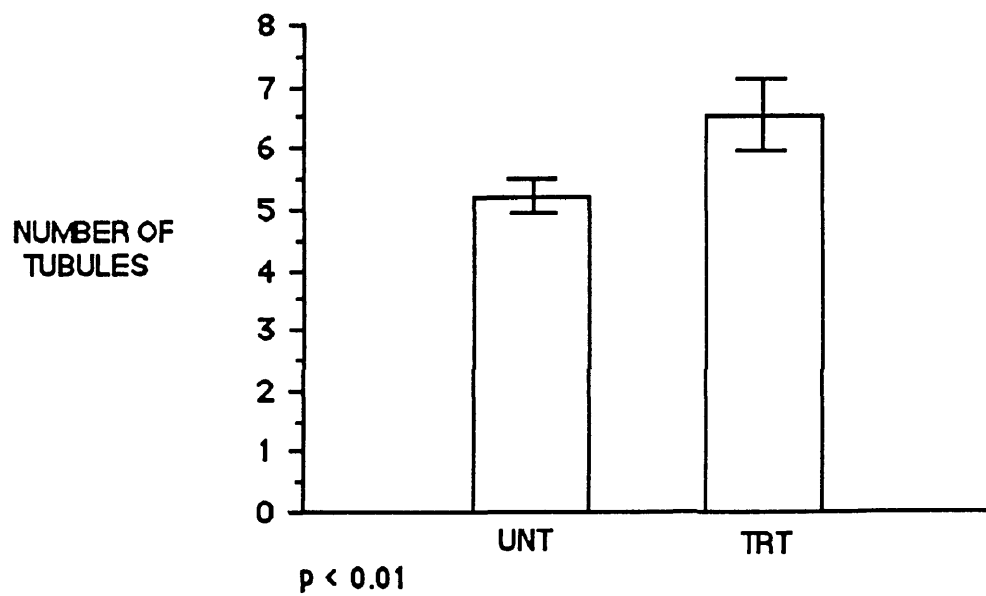


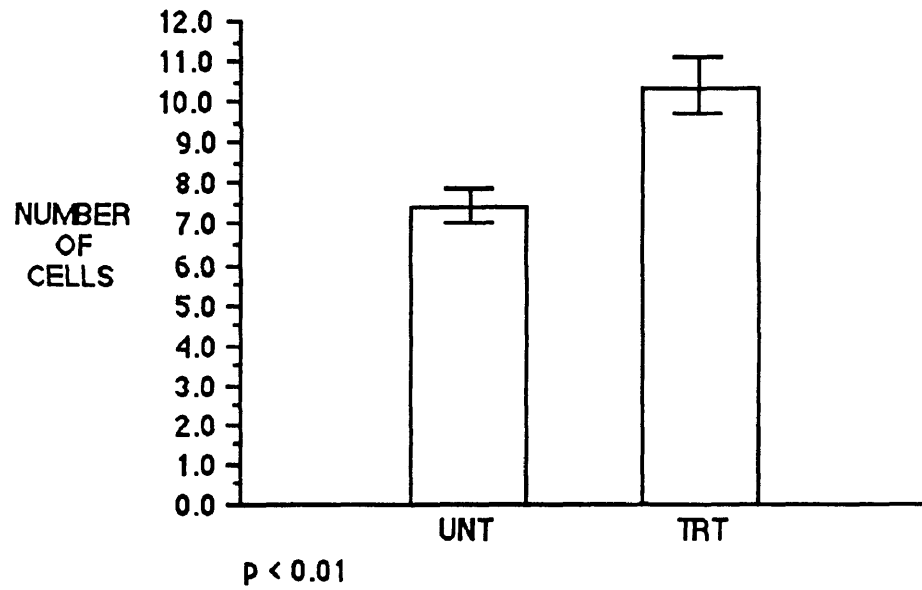
Figure 16: Mean number of cells per seminiferous tubule (with minor/major axis ratio >0.85) along the transect. Mean values represent numbers of cells which lie on the major axis of the seminiferous tubules. Populations three and four are combined. Error bars represent \pm S.E.M.

Figure 17: Mean number of spermatids with acrosome formation per seminiferous tubules (with minor/major axis ratio >0.85) along transect.

Mean values represent numbers of spermatids with acrosomes which lie on the major axis of the seminiferous tubules. Populations three and four are combined.

Error bars represent \pm S.E.M.

MEAN NUMBER OF CELLS PER
SELECTED SEMINIFEROUS TUBULE



MEAN NUMBER OF SPERMATIDS WITH
ACROSOME FORMATION

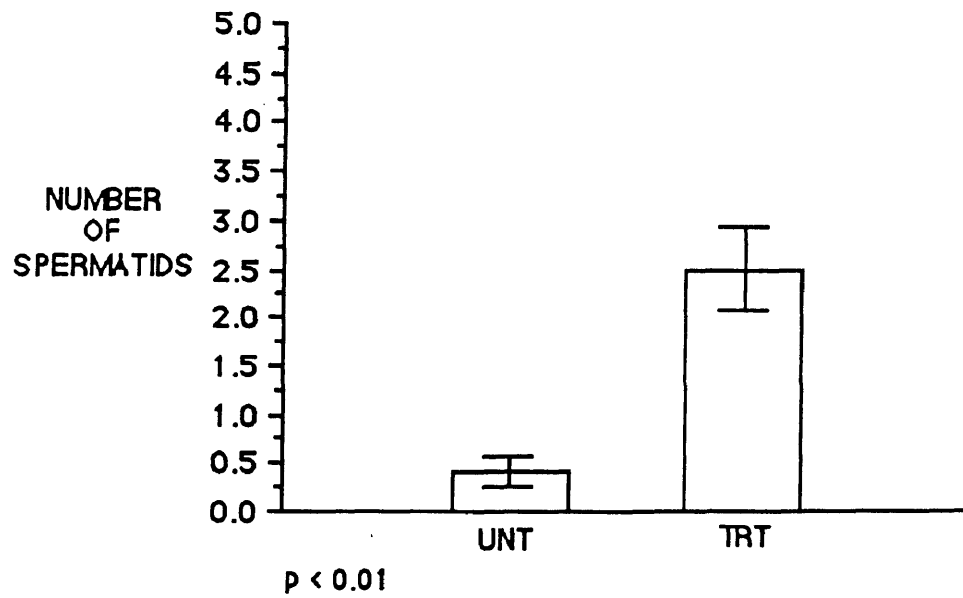
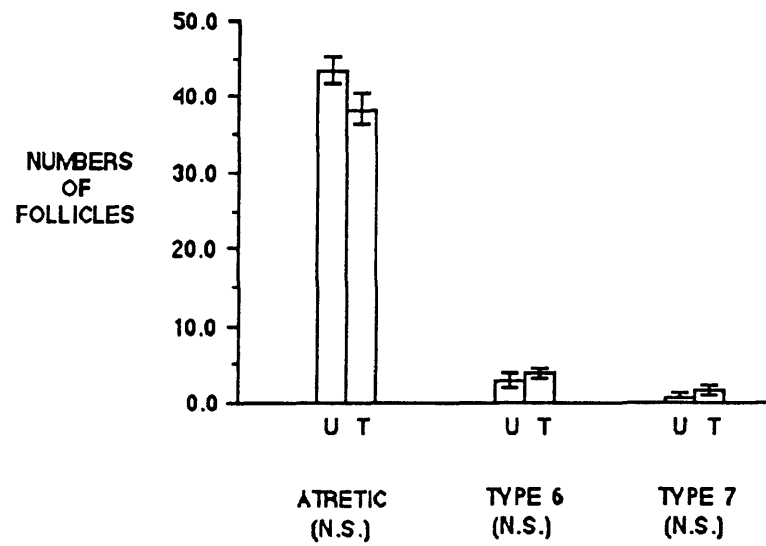


Figure 18: Mean number of atretic, type six, type seven, and type eight follicles as well as mean number of corpora lutea. Error bars represent \pm S.E.M.

Figure 19: Mean number of type eight follicles and corpora lutea. Error bars represent \pm S.E.M.

MEAN NUMBER OF ATRETIC,
TYPE SIX, AND TYPE SEVEN FOLLICLES



MEAN NUMBER OF TYPE EIGHT FOLLICLES
AND CORPORA LUTEA

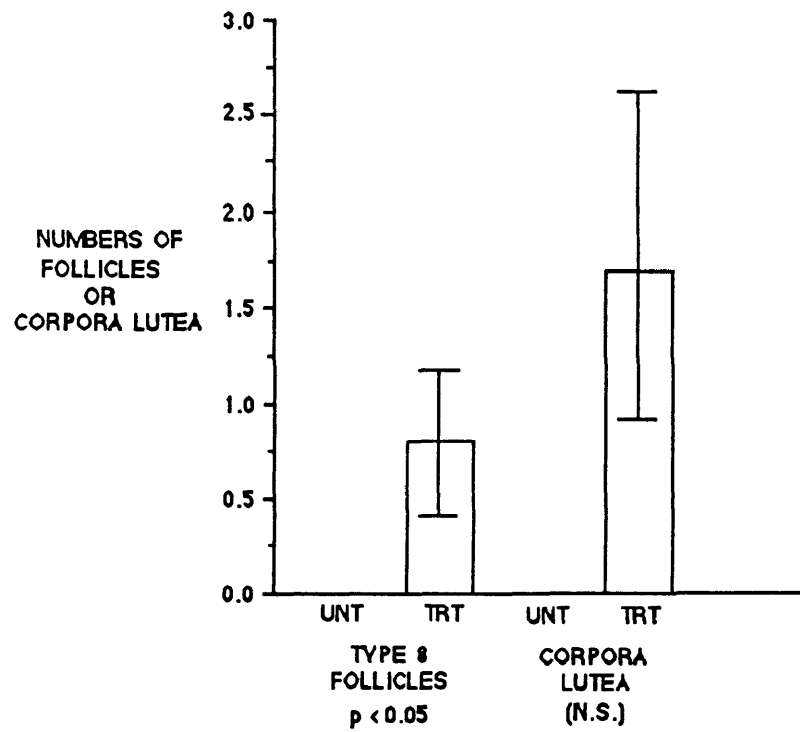


Figure 20: Photomicrograph of an untreated male seminiferous tubule cross-section. Spermatogonia (SG) along with primary (PS) spermatocytes are present. Note the few spermatids (SP) which lack acrosome development. (X 1056).

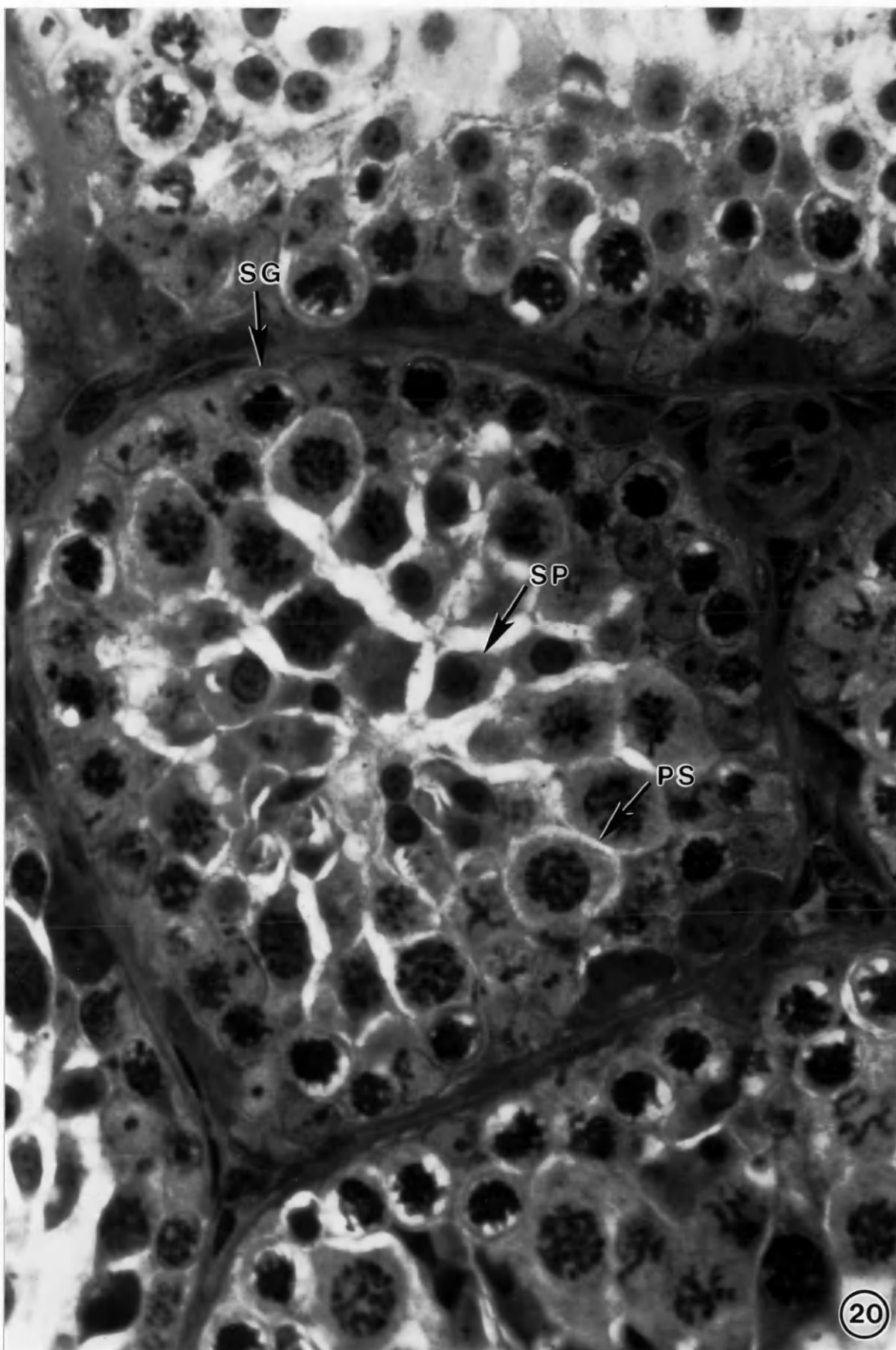


Figure 21: Photomicrograph of a treated male seminiferous tubule cross-section. Spermatogonia (SG) along with primary (PS) and secondary (SS) spermatocytes are present. Note the relative abundance of spermatids (SP) with developing and mature acrosome formation. (X 1360).

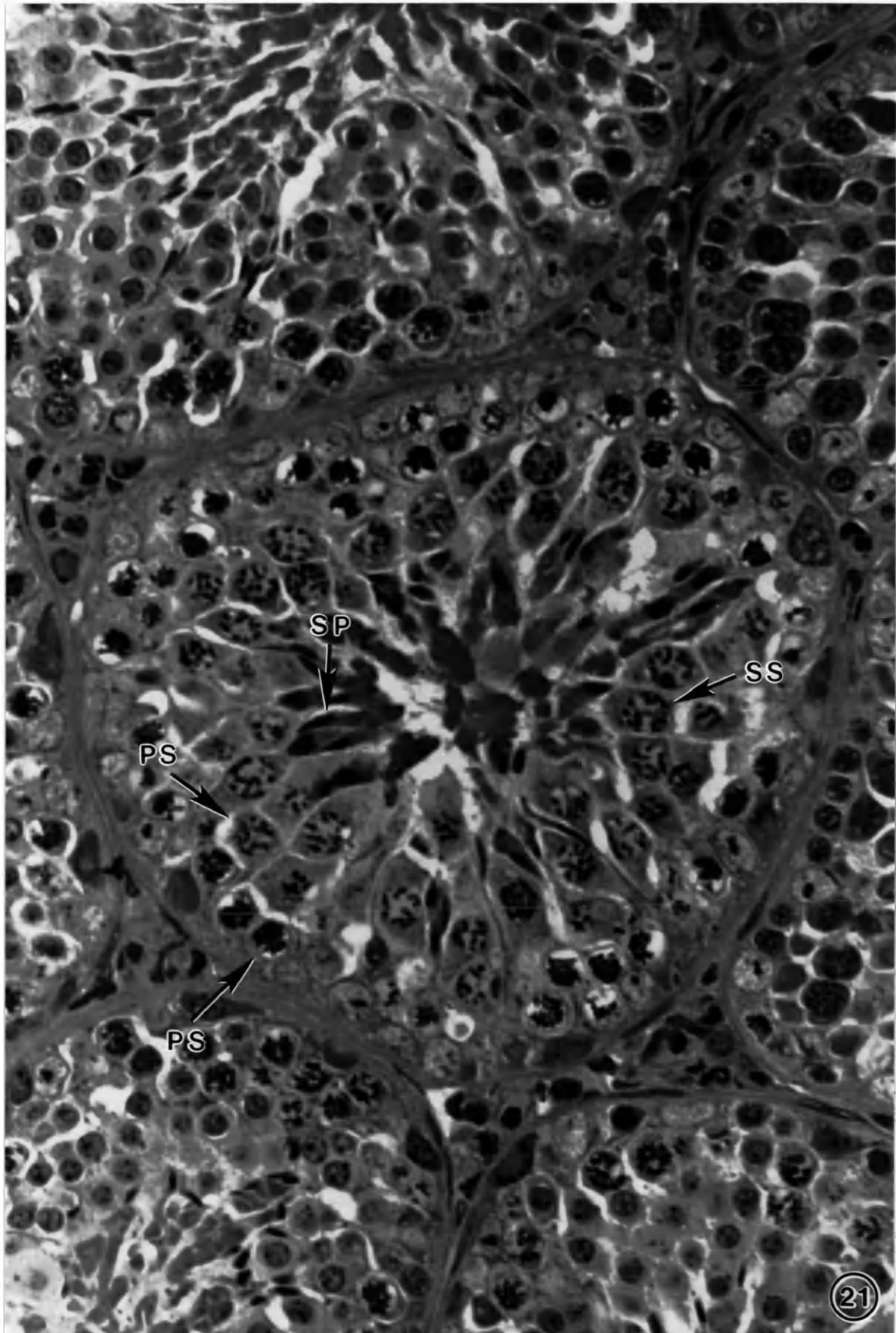


Figure 22: Photomicrograph of an untreated female ovary cross-section. Atretic (AT), type 3a (3A), type 5a (5A), and type 5b (5B) follicles are present. (X 304).

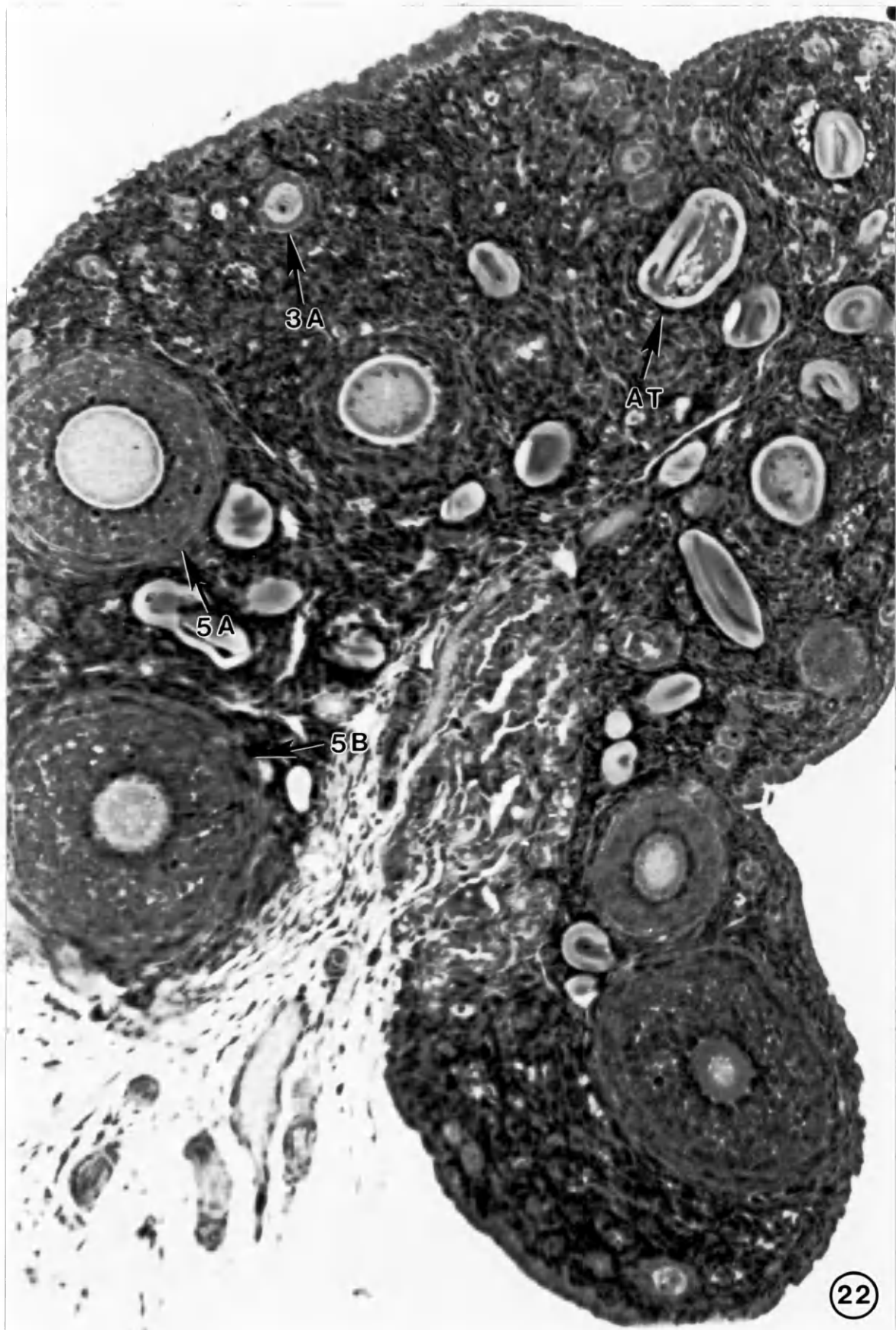
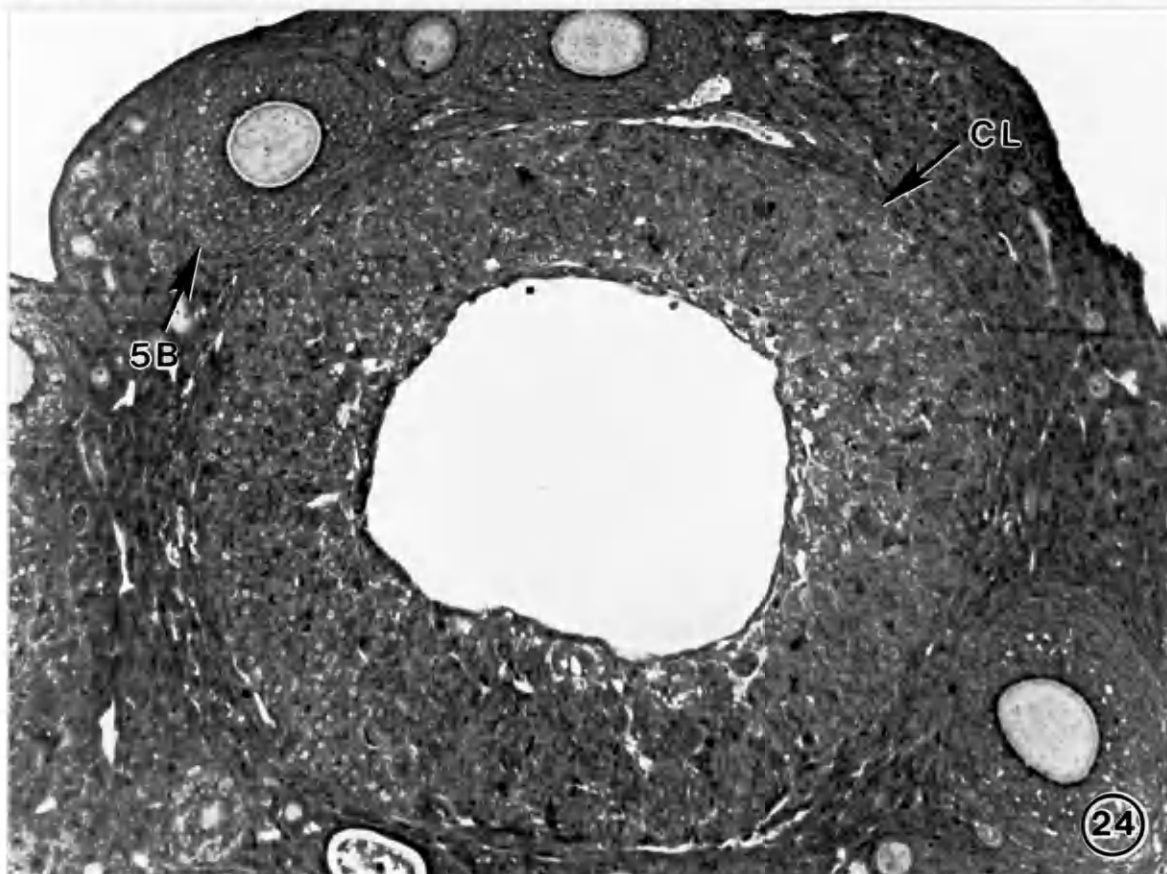
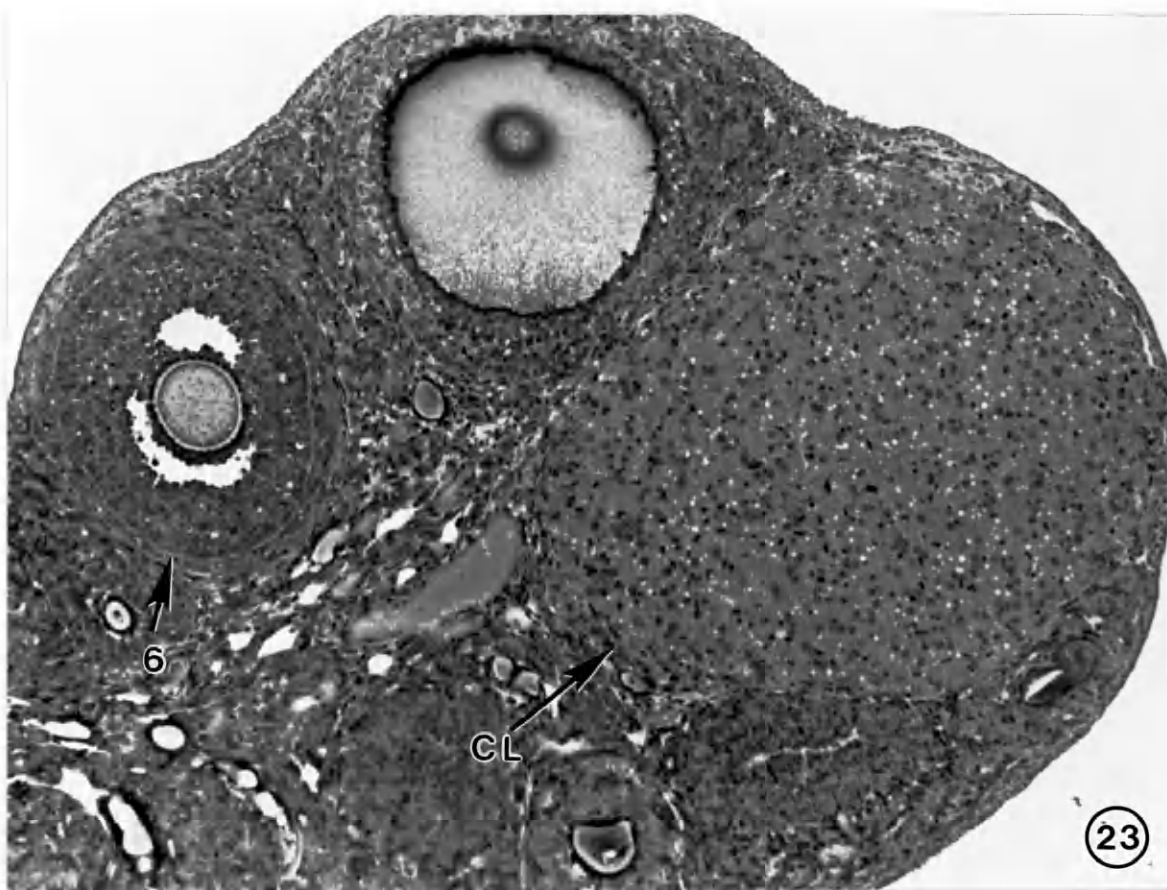


Figure 23: Photomicrograph of a treated female ovary cross-section. Note the type 6 (6) and corpus luteum (CL). (X 224).

Figure 24: Photomicrograph of a treated female ovary cross-section. Note the prescence of type 5b (5B) follicles and corpus luteum (CL) with lumen, indicating recent ovulation. (X 224).



DISCUSSION

Serum T3 concentrations as determined by radioimmunoassay were highly significantly ($P < 0.001$) elevated in both treated males and females as compared with untreated males and females (Tables 3 and 4, and Figures 4 and 5). The radioimmunoassay of T3 was validated based on the evidence that the serial dilutions of P. maniculatus serum produced a response-to-dilution curve with a slope that was not significantly different from a corresponding serial dilution of authentic standard T3 (Figures 1 and 2). This established that the binding properties of P. maniculatus T3 and human T3 supplied with the kit were statistically equivalent. T3 was the thyroid hormone chosen to be administered because it is the most biologically active thyroid hormone (Lerman, 1953; Chopra, 1986). T3 is produced via direct synthesis and secretion of the thyroid gland as well as peripheral conversion of T4 (Schwartz et al., 1971; Fischer et al., 1972). Current data indicates that one third to one half of T4 is converted to T3 (Chopra, 1986). Whether produced directly by the thyroid gland or

by peripheral conversion, T₃ is considered to be responsible for 85% of the overall biological effects of thyroid hormones (Chopra, 1986). The remaining 15% is attributed to the intrinsic activity of T₄ (Chopra, 1986).

The mean per capita food consumption in both populations was highly significantly ($P < 0.0001$) elevated during T₃ administration as compared with the period prior to hormone administration (Figure 7). These results are in accordance with reported T₃ effects on both basal metabolic rate and calorogenesis in rats (Frieden and Lipner, 1971; Becker, 1986; Shambaugh, 1986). It is probable that the increased per capita food consumption observed in this study was due to an increase in basal metabolic rate caused by elevated serum concentrations of T₃. Since the per capita food consumption of both populations was significantly elevated for day 5 through day 35 of the treatment period it is probable that serum T₃ concentrations were also elevated throughout the period.

The body fat of treated males and females tended to be reduced in comparison with the corresponding values for untreated males ($P = 0.146$) and females ($P = 0.089$) (Tables 1 and 2, and Figures 8 and 9). T₃ increases oxidative metabolic processes which in turn cause

depletion of carbohydrate stores (Shambaugh, 1986; Sterling, 1986). When carbohydrate stores are depleted, free fatty acids are mobilized from adipose tissue to the liver where gluconeogenesis occurs (Challoner, 1969; Loeb, 1986). Thus, it is probable that the concentrations of exogenous T3 administered in this study increased the rates of the various metabolic processes to such a degree that adipose tissue deposits were reduced.

This study provided evidence that exogenous T3 causes gravimetric change of reproductive organ weights. Mean treatment male testis and seminal vesicle weights were significantly ($P < 0.05$) greater than mean untreated male values (Table 1, and Figures 10 and 12). Although statistically significant, the mean weights of testes and seminal vesicles in treated males attained levels that were only 25% and 15%, respectively, of mean values reported for reproductively capable males (Bradley and Terman, 1981c; Terman, 1969). These weight increases may be due to an elevation of serum T3 to normal or supranormal levels in animals that had previously been in hypothyroid states. When hypothyroidism was induced in immature male rats by thyroidectomy or thiouracil administration, testis and seminal vesicle weights were significantly lower than controls (Maqsood and Reineke, 1950; Kalland et al., 1978). Reproductively inhibited animals selected from laboratory populations of prairie

deermice have been shown to have low serum T4 and T3 concentrations concomittant with a signnificant reduction in testis and seminal vesicle weights (Peebles, 1981; Pitman, 1983).

The increased testis and seminal vesicle weights in the current study can be attributed to the effect of T3 on androgen metabolism. Hypothyroidism increases metabolic clearance rates of androgens thus reducing serum levels of testosterone (Christy, 1986). Exogenous T3 may reduce the clearance rates of androgens and thereby increase serum levels of testosterone. Hypothyroidism may also be associated with and possibly responsible for reduced serum levels of prolactin (Bakke et al., 1970; Sowers et al., 1980; Wark and Gurtler, 1986). Subsequently, hypoprolactinemia may be a contributing factor in the reduction of gonadal LH receptors, thereby reducing the synthesis and secretion of testosterone (Kirkland and Bradley, 1986). A reduction in testosterone as evidenced in reproductively inhibited population males (Bradley and Terman, 1981b) may be partially responsible for reduced seminal vesicle and testis weights as well as reduced spermatogenesis. Exogenous T3 may restore normal prolactin secretion and thereby stimulate some recovery of testosterone synthesis and metabolism as well as a concomittant increase in testis and seminal vesicle weights.

Mean ovarian and uterine weights were significantly ($P < 0.05$ and $P < 0.001$ respectively) increased in treated females as compared with untreated females (Table 4, and Figures 11 and 13). Although statistically significant, the mean weights of ovaries and uteri in treated females reached levels that were only 30% and 20%, respectively, of the mean values reported for reproductively capable females (Terman, 1969; Bradley and Terman, 1981c). This weight increase may be due in part to the elevation of T3 serum concentrations to normal or supranormal levels in previously hypothyroid individuals. Hypothyroidism induced by thyroidectomy causes a reduction in ovarian and uterine weights in rats (Hagino, 1971; Jones and Tracy, 1974; Hendrich et al., 1976; Norris, 1985) and gerbils (Dixit et al., 1976). Pitman (1983) reported that significantly reduced ovarian weights are correlated with hypothyroidism exhibited by prairie deermice.

If the degree of hypothyroidism demonstrated in reproductively inhibited population females does influence reductions in ovarian and uterine weights then exogenous thyroid hormone administration may bring about an increase in ovarian weights. Hendrich et al., (1976) demonstrated that exogenous T4 administration increased ovarian weights in adult rats. Past work by Peebles et al. (1984) with prairie deermice demonstrated that T4 feeding produced a degree of ovarian and uterine weight

increase intermediate to values of inhibited and reproductively capable females. The current study has also shown an increase in ovarian and uterine weights of adult prairie deermice after T3 administration (Table 4).

In addition to causing weight increases in reproductive organs, thyroid hormones can also effect testicular histology. Hypothyroidism induced by thiouracil treatment in male rabbits caused a halt in testicular interstitial cell development (Magsood, 1951). Similarly, Amin and el-Sheikh (1977) found the same phenomenon to occur in thiouracil treated male rats. Dixit et al. (1976) reported a decrease in spermatogenesis in thyroidectomized male gerbils. Reproductively inhibited male prairie deermice are characterized by both hypothyroidism (Peebles et al., 1984; Pitman and Bradley, 1984) and decreased spermatogenesis (Bradley and Terman, 1981c).

The current study provided histological evidence that exogenous T3 administration caused some degree of histological change in terms of male and female gametogenesis. All of the testicular histological parameters analyzed showed significant increases in treated males (Table 5, and Figures 14 and 15). The mean testis width and mean number of seminiferous tubules (with minor/major axis ratio >0.85) were both

significantly ($P < 0.01$) increased in treated males. The mean number of cells per selected seminiferous tubule and the mean number of spermatids with acrosome formation were also significantly ($P < 0.01$) elevated in treated males (Table 5, and Figures 16 and 17). Of all the histological parameters studied in males, these last two are the most important in distinguishing the level of reproductive recovery. Past studies by Maqsood and Reineke (1950) showed that administration of thyroprotein initiated spermatogenesis in immature male mice. Maqsood (1951) found that physiological doses of L-thyroxine in immature rabbits stimulated increases in the numbers of testicular interstitial cells, and the numbers and activity of spermatogenic cells. Amin and el-Sheikh (1977) also found that administration of L-thyroxine in rats caused an increase in the size and number of testicular spermatogenic and interstitial cells. Peebles et al. (1984) found significant increases in testis width, mean number of seminiferous tubules, total number of cells on transect, and number of spermatids with acrosome formation in male prairie deermice injected with T4. As previously mentioned, these recovery effects may have also been influenced by repeated daily handling of T4 injected reproductively inhibited population animals. In the current study the changes in testicular histology may be the result of increased serum testosterone levels

produced by the T3 mediated effects previously mentioned (Christy, 1986).

Conditions of hypothyroidism are also linked to degenerative changes in ovarian histology. Hagino (1971) reported a decrease in the number of ova in thyroidectomized rats. Dixit et al. (1976) found that thyroidectomy of female gerbils caused a decrease in the number of corpora lutea and a subsequent increase in atretic follicle numbers. An immature ovarian condition is also exhibited by reproductively inhibited prairie deer mice. This condition is characterized by significantly reduced numbers of Type 8 follicles and corpora lutea as well as significantly increased numbers of atretic follicles (Terman, 1973a; Bradley and Terman, 1981c).

Ovarian histology was apparently affected by exogenous T3 administration. Although the mean numbers of atretic, Type 6 and Type 7 follicles as well as corpora lutea were not significantly different between the two treatment groups, the mean number of Type 8 follicles was significantly ($P < 0.04$) increased in treated females (Table 6, and Figures 18 and 19). Such results suggest that T3 may have stimulated the development of Type 8 follicles yet did not promote the final process of ovulation in a statistically significant number of T3

treated females. This limited degree of follicular development is possibly based on the role of T3 as a biological amplifier of FSH actions. Maruo et al., (1987) reported that T3 and FSH administration to cultured porcine granulosa cells caused significant increases in morphological differentiation, LH/humanCG receptor formation, and steroidogenic enzyme (3 beta-hydroxysteroid and aromatase) induction. All of these effects, which resulted in significantly increased secretion of progesterone and estrogen, were only observed in granulosa cells harvested from "small" follicles. With respect to these findings, it may be that in the current study the elevated levels of FSH in inhibited population females (Bradley and Terman, 1981c) along with the exogenous T3 caused a development of "small" Type 6 and 7 follicles into Type 8 follicles without ovulation and subsequent corpus luteum formation.

The phenomenon of reproductive inhibition exhibited by laboratory populations of prairie deermice is probably influenced by the function of the pituitary-adrenal and the pituitary-thyroid axes in response to environmental pressures. Such function is probably initiated by external stimuli, such as social stress, and further compounded due to the interrelated nature of the pituitary-adrenal and the pituitary-thyroid axes.

Chronic social stress in laboratory population prairie deermice could be brought about by continuous interaction with other animals in a limited space. Chronic stress due to overcrowding has been reported (Christian, 1950, 1971a, 1971b, 1975, 1980) to cause elevated secretion of ACTH and subsequent elevation of corticosteroid secretion by the adrenal cortex in some rodent species. Christian has proposed that this hyperfunctioning of the pituitary-adrenal axis is the primary cause of delayed maturation in house mice (Christian, 1964, 1971b) and prairie deermice (Christian, 1971b).

Christian reported that ACTH may directly affect reproductive function by inhibiting pituitary releases of gonadotropins. Christian has also proposed that ACTH induced elevated serum levels of corticosteroids as well as ACTH itself may have direct inhibiting affects on gonads (Christian, 1964, 1978). ACTH has also been linked to decreased plasma testosterone levels (Doerr and Pirke, 1975; Saez et al., 1977) as well as decreased testosterone synthesis and secretion (Kim et al., 1978; Magrini et al., 1978). This reduction in testosterone level may be due to the ability of ACTH to compete with LH for binding sites on Leydig cells (Beitins et al., 1973). Besides affecting testicular endocrine function, ACTH has also been reported to cause degenerative changes

in testis histology (Kapil et al., 1978). Reproductive inhibition in laboratory populations of prairie deermice may not be directly affected by ACTH based on reports that neither ACTH concentrations are elevated nor are adrenals hypertrophied in reproductively inhibited population animals (Bradley and Terman, 1981; Coppes and Bradley, 1984).

Although much evidence supports the inhibitory affects of ACTH on reproductive function, there are also reports linking disrupted reproductive function with glucocorticoids. It has been found that high levels of glucocorticoids are associated with decreased synthesis and secretion of testosterone (Doerr and Pirke, 1975; Schaison et al., 1978; nim et al., 1978; Magrini et al., 1978). Luton et al. (1977) postulated that glucocorticoids affected testosterone levels by inhibiting pituitary LH secretion. However, Schaison et al. (1978) and McKenna et al. (1979) found no reduction in plasma LH associated with high concentrations of glucocorticoids. As a result, Schaison et al. (1978) and McKenna et al. (1979) have proposed that glucocorticoids may have a direct inhibiting effect on testes. These proposals are supported by reports of glucocorticoid interference with cholesterol side chain cleavage (Saez et al., 1977), as well as interference of testicular DNA and protein synthesis (Evain et al., 1976; Saez et al.,

1977). Glucocorticoids have also been shown to inhibit the synthesis and secretion of prolactin via a reduction of prolactin gene expression (Leung et al., 1980; Wark and Gurtler, 1986). The resulting hypoprolactinemia may contribute to a reduction in testicular LH receptors thereby promoting reproductive inhibition (Kirkland and Bradley, 1986). Such speculation may explain the apparent discrepancies between normal serum LH levels and reduced testosterone concentrations in reproductively inhibited male prairie deer mice. Since it is known that corticosterone levels are significantly elevated in prairie deer mice (Bradley and Terman, 1981a), it is possible that the reproductive inhibition in population animals is in part influenced by the effects of glucocorticoids.

In addition to the direct effects that glucocorticoids have on reproductive function, they may also initiate changes in the physiology of the pituitary-thyroid axis and a reduction in peripheral conversion of T₄ to T₃. It is known that elevated concentrations of serum corticosterone cause decreases in serum thyroxine and T₃ (Kuhl and Ziff, 1952; Williams et al., 1975). This effect has been reported to be the result of the inhibition of thyroid releasing hormone (thyrotropin or TRH) secretion by corticosterone (Retiene et al., 1968; Wilber and Uttinger, 1969; Tolis et al., 1974; Pamenter

and Hedge, 1980). Such a mechanism could cause a reduction in the synthesis and secretion of thyroid hormones possibly leading to a hypothyroid state. More recently stress-induced elevation of plasma glucocorticoids in rats has been reported to reduce the peripheral conversion of T₄ to T₃ in liver and kidney (Bianco et al., 1987). This reduction is believed to be the result of glucocorticoids inhibitory effects on the production of 5'-deiodinase (Chopra et al., 1975; Balsam and Ingbar, 1978; Decuyper et al., 1983; McCann et al., 1984). Since peripheral conversion of T₄ is an important source of plasma T₃, such an effect mediated by glucocorticoids may also be responsible for reduced levels of T₃ in reproductively inhibited population animals.

Whether the hypothyroidism exhibited by reproductively inhibited population animals is initiated by a hyperfunctional pituitary-adrenal axis or directly by environmental factors working through the pituitary-thyroid axis, it is a condition which may directly inhibit reproductive organ development and also propagate alterations of adrenal physiology. Thyroid deficiency may disrupt the normal transcription of functional proteins in reproductive tissues (Crispell et al., 1956). Moreover, reduced thyroid hormone levels may alter the ability of reproductive tissue to respond to

gonadotropins (Turner and Bagnara, 1976). It could be that exogenous T3 can reverse these conditions by effects mediated directly at the target tissue level.

Hypothyroidism may also affect reproductive function via several indirect influences. The effects of hypothyroidism on pituitary gonadotropin release is well documented and yet controversial. Hypothyroidism induced in male rats by thyroidectomy has been reported to either decrease serum LH and FSH (Bruni et al., 1975; Wong, 1980) or leave them unaffected (Kalland et al., 1978). There is a report by Chu (1944b) that found thyroidectomized rabbits had increased serum levels of FSH and decreased levels of LH. Population female deermice are known to have elevated FSH and decreased LH concentrations relative to controls. Therefore, Chu's findings may provide some evidence for a thyroid mediated influence on gonadotropin secretion in prairie deermice. Exogenous T3 may initiate a recovery of normal gonadotropin secretion which, in turn, may restore the normal pre-ovulatory LH surge which is believed to be absent in inhibited population females (Bradley and Terman, 1981c).

Subnormal thyroid levels may also affect the pituitary synthesis and secretion of growth hormone. (Kikuyama et al., 1974; Augustine and Hymer, 1978; Ciori

et al., 1979; Bernal and DeGroot, 1980; Wong et al., 1980). The action of T3 on GH synthesis has been reported to occur at the level of GH mRNA transcription (Wood et al., 1986). Wood et al., (1986) reported that T3 replacement in hypothyroid rats rapidly stimulated GH mRNA transcription followed by gradual restoration of pituitary and serum GH concentrations. Thyroid hormone is also known to have a synergistic effect in relation to GH metabolism. Both reduced body weights and reproductive organ weights could be explained by the influence of hypothyroidism on GH synthesis, secretion and action.

Conditions of hypothyroidism are also associated with: the reduced clearance of serum adrenocorticoids; an increase in corticosterone binding globulin; and an increase in adrenal sensitivity to ACTH stimulation (Zarrow et al., 1957; McCarthy et al., 1959; Steinetz and Beach, 1962; D'Agostino and Henning, 1982). All of these factors would lead to increased serum corticosterone levels without a concomitant increase in ACTH levels or adrenal hypertrophy. This might, in part, explain why neither serum levels of ACTH were significantly elevated nor were adrenals hypertrophied in inhibited male prairie deer mice (Coppes and Bradley, 1984) even though corticosterone levels were significantly increased in both sexes of inhibited animals. If this mechanism were

to contribute to increases in serum corticosterone then it may further promote the original suppression of TSH and subsequent thyroid function in population males. Increased serum corticosterone concentrations via this mechanism may have direct inhibiting effects on reproductive organ function. Administration of exogenous T3 may therefore increase the clearance and metabolism of glucocorticoids and thereby reduce elevated serum levels of corticosterone in population animals. Thus, T3 may indirectly initiate physiological and histological changes by reducing the negative influence which elevated glucocorticoid levels are known to have on reproductive function.

Although thyroid function may have a role in the reproductive inhibition exhibited by prairie deer mice from laboratory populations, this role is probably in concert with the functions of other endocrine systems. This view is supported by the evidence from the current study that administration of exogenous T3 alone initiates significant, but rather limited, degrees of gravimetric and histological change. It may be that the duration of administration and the dose of T3 used in the current study were inadequate. However, this suggestion must be viewed in light of reports that 50% of reproductively inhibited population animals reproduce within 30 days of removal from populations and pairing with reproductively

proven mates (Terman, 1973a). Further work concerning the role of thyroid hormone in the reproductive inhibition of laboratory populations of prairie deermice should attempt to discern possible changes in serum levels of LH, FSH, GH, prolactin, corticosterone, estrogen, and testosterone that may be the result of exogenous T3 administration at various doses and durations.

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